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(54) Title: NOVEL POTASSIUM CHANNELS AND GENES ENCODING THESE POTASSIUM CHANNELS

(57) Abstract: This invention relates to novel potassium channels and genes encoding these channels. More specifically the invention provides isolated polynucleotides encoding the KCNQ5 potassium channel subunit, cells transformed with these polynucleotides, transgenic animals comprising genetic mutations, and the use of the transformed cells and the transgenic animals for the *in vitro* and *in vivo* screening of chemical compounds affecting KCNQ5 subunit containing potassium channels.

NOVEL POTASSIUM CHANNELS AND GENES ENCODING THESE POTASSIUM CHANNELS

TECHNICAL FIELD

This invention relates to novel potassium channels and genes encoding these channels. More specifically the invention provides isolated polynucleotides encoding the KCNQ5 potassium channel subunit, cells transformed with these polynucleotides, transgenic animals comprising genetic mutations, and the use of the transformed cells and the transgenic animals for the *in vitro* and *in vivo* screening of chemical compounds affecting KCNQ5 subunit containing potassium channels.

BACKGROUND ART

Potassium channels participate in the regulation of electrical signalling in excitable cells, and regulates the ionic composition of biological fluids. Mutations in the four known genes of the *KCNQ* branch of the K^+-channel gene family underlie inherited cardiac arrhythmia's, in some cases associated with deafness, neonatal epilepsy, and the progressive hearing loss of the elderly (presbyacusis).

Ion channels play important roles in signal transduction and in the regulation of the ionic composition of intra- and extracellular fluids. KCNQ1 is a typical member of the voltage-gated potassium channel superfamily with 6 transmembrane domains and a pore region situated between the fifth and the sixth transmembrane domain. The minK protein (also known as KCNE1 or IsK) has a single transmembrane span and cannot form potassium channels on its own. However, as a β-subunit it enhances and modifies currents mediated by KCNQ1. These heteromeric channels participate in the repolarization of the heart action potential. Certain mutations in either *KCNQ1* or *KCNE1* cause a form of the autosomal dominant long QT syndrome (LQTS), a disease characterised by repolarization anomalies of cardiac action potentials resulting in arrhythmias and sudden death. Interestingly, other mutations in either gene lead to the recessive Jervell and Lange-Nielsen (JLN) syndrome that combines LQTS with congenital deafness. In order to cause deafness, KCNQ1/minK currents must be reduced below levels that are already sufficiently low to cause cardiac arrhythmia.

Mutated and non-mutated KCNQ2 and KCNQ3 potassium channels have been disclosed in WO 99/07832, WO 99/21875 and WO 99/31232.

SUMMARY OF THE INVENTION

We have now cloned and characterised KCNQ5, a novel member of the KCNQ family of potassium channel proteins. KCNQ5 forms heteromeric channels with other KCNQ channel subunits, in particular KCNQ3 and KCNQ4.

The present invention has important implications for the characterisation and exploitation of this interesting branch of the potassium channel super family.

Accordingly, in its first aspect, the invention provides an isolated polynucleotide having a nucleic acid sequence which is capable of hybridising under at least medium stringency conditions with the polynucleotide sequence presented as SEQ ID NO: 1, its complementary strand, or a sub-sequence thereof.

In another aspect the invention provides a recombinantly produced polypeptide encoded by the polynucleotide of the invention.

In a third aspect the invention provides a cell genetically manipulated by the incorporation of a heterologous polynucleotide of the invention.

In a fourth aspect the invention provides a method of screening a chemical compound for inhibiting or activating or otherwise modulating the activity on a potassium channel comprising at least one KCNQ5 channel subunit, which method comprises the steps of subjecting a KCNQ5 channel subunit containing cell to the action of the chemical compound; and monitoring the membrane potential, the current, the potassium flux, or the secondary calcium influx of the KCNQ5 channel subunit containing cell.

In a fifth aspect the invention relates to the use of a polynucleotide sequence of the invention for the screening of genetic materials from humans suffering from neurological diseases for mutations in the *KCNQ5* gene.

In a sixth aspect the invention relates to the chemical compound identified by the method of the invention, in particular to the use of such compounds for diagnosis, treatment or alleviation of a disease related to diseases or adverse conditions of the CNS, including affective disorders, Alzheimer's disease, anxiety, ataxia, CNS damage caused by trauma, stroke or neurodegenerative illness, cognitive deficits, compulsive behaviour, dementia, depression, Huntington's disease, mania, memory impairment, memory disorders, memory dysfunction, motion disorders, motor disorders, neurodegenerative diseases, Parkinson's disease and Parkinson-like motor disorders, phobias, Pick's disease, psychosis, schizophrenia, spinal cord damage, stroke, tremor, seizures, convulsions and epilepsy.

In a seventh aspect the invention provides a transgenic animal comprising a knock-out mutation of the endogenous *KCNQ5* gene, a replacement by or an additional expression of a mutated *KCNQ5* gene, or genetically manipulated in order to over-express the *KCNQ5* gene or to over-express mutated *KCNQ5* gene.

In an eighth aspect the invention relates to the use of the transgenic animal of the invention for the *in vivo* screening of therapeutic compounds.

Other objects of the invention will be apparent to the person skilled in the art from the following detailed description and examples.

DETAILED DISCLOSURE OF THE INVENTION

The present invention provides novel potassium channels and genes encoding these channels. The invention also provides cells transformed with these genes, transgenic animals comprising genetic mutations, and the use of the transformed cells and the transgenic animals for the *in vitro* and *in vivo* screening of drugs affecting KCNQ5 containing potassium channels.

Polynucleotides

In its first aspect, the invention relates to novel nuceic acid molecules encoding a polypeptide comprising all or a portion of a KCNQ5 protein.

In a preferred embodiment, the polynucleotides of the invention are such which have a nucleic acid sequence capable of hybridising under at least medium stringency conditions with the polynucleotide sequence presented as SEQ ID NO: 1, its complementary strand, or a sub-sequence thereof.

The polynucleotides of the invention include DNA, cDNA and RNA sequences, as well as anti-sense sequences, and include naturally occurring, synthetic, and intentionally manipulated polynucleotides. The polynucleotides of the invention also include sequences that are degenerate as a result of the genetic code.

As defined herein, the term "polynucleotide" refers to a polymeric form of nucleotides of at least 10 bases in length, preferably at least 15 bases in length. By "isolated polynucleotide" is meant a polynucleotide that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. The term therefore includes recombinant DNA which is incorporated into an expression vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule, e.g. a cDNA, independent from other sequences.

The polynucleotides of the invention also include allelic variants and "mutated polynucleotides" having a nucleotide sequence that differs from the sequence presented as SEQ ID NO: 1 at one or more nucleotide positions. The mutated polynucleotide may in particular be a polynucleotide of the invention having a nucleotide sequence as in SEQ ID NO: 1, which sequence, however, differs from SEQ ID NO: 1 so as to effect the expression of a variant polypeptide. The mutated polynucleotide may be

a polynucleotide of the invention having a nucleotide sequence encoding a potassium channel having an amino acid sequence that has been changed at one or more positions. The mutated polynucleotide may in particular be a polynucleotide of the invention having a nucleotide sequence encoding a potassium channel having an amino acid sequence that has been changed at one or more positions located in the conserved regions, as defined by Table 1, below.

Hybridisation Protocol

The polynucleotides of the invention are such which have a nucleic acid sequence capable of hybridising with the polynucleotide sequence presented as SEQ ID NO: 1, its complementary strand, or a sub-sequence thereof, under at least medium, medium/high, or high stringency conditions, as described in more detail below.

In a preferred embodiment the polynucleotide is a fragment of at least 15 bases in length which is sufficient to permit the fragment to hybridise to DNA that encodes a polypeptide of the invention, preferably the polypeptide having the amino acid sequence presented as SEQ ID NO: 2 under at least medium, medium/high, or high stringency conditions, as described in more detail below.

Suitable experimental conditions for determining hybridisation between a nucleotide probe and a homologous DNA or RNA sequence, involves pre-soaking of the filter containing the DNA fragments or RNA to hybridise in 5 x SSC [Sodium chloride/Sodium citrate; cf. *Sambrook et al.*; Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Lab., Cold Spring Harbor, NY 1989] for 10 minutes, and pre-hybridisation of the filter in a solution of 5 x SSC, 5 x Denhardt's solution [cf. *Sambrook et al.; Op cit.*], 0.5 % SDS and 100 µg/ml of denatured sonicated salmon sperm DNA [cf. *Sambrook et al.; Op cit.*], followed by hybridisation in the same solution containing a concentration of 10 ng/ml of a random-primed [*Feinberg A P & Vogelstein B*; Anal. Biochem. 1983 **132** 6-13], ^{32}P-dCTP-labeled (specific activity > 1 x 10^9 cpm/µg) probe for 12 hours at approximately 45°C.

The filter is then washed twice for 30 minutes in 2 x SSC, 0.5 % SDS at a temperature of at least at least 60°C (medium stringency conditions), preferably of at least 65°C (medium/high stringency conditions), more preferred of at least 70°C (high stringency conditions), and even more preferred of at least 75°C (very high stringency conditions).

Molecules to which the oligonucleotide probe hybridises under these conditions may be detected using a x-ray film.

DNA Sequence Homology

In a preferred embodiment, the polynucleotides of the invention show a homology of at least 65%, preferably at least 70%, more preferred at least 80%, even more preferred at least 90%, most preferred at least 95%, with the polynucleotide sequence presented as SEQ ID NO: 1.

As defined herein, the DNA sequence homology may be determined as the degree of identity between two DNA sequences indicating a derivation of the first sequence from the second. The homology may suitably be determined by means of computer programs known in the art such as GAP provided in the GCG program package [*Needleman S B and Wunsch C D*, Journal of Molecular Biology 1970 **48** 443-453] using default parameters suggested herein.

Cloned Polynucleotides

The isolated polynucleotide of the invention may in particular be a cloned polynucleotide.

As defined herein, the term "cloned polynucleotide", refers to a polynucleotide or DNA sequence cloned in accordance with standard cloning procedures currently used in genetic engineering to relocate a segment of DNA, which may in particular be cDNA, i.e. enzymatically derived from RNA, from its natural location to a different site where it will be reproduced.

Cloning may be accomplished by excision and isolation of the desired DNA segment, insertion of the piece of DNA into the vector molecule and incorporation of the recombinant vector into a cell where multiple copies or clones of the DNA segment will be replicated, by reverse transcription of mRNA (reverse transcriptase technology), and by use of sequence-specific oligonucleotides and DNA polymerase in a polymerase chain reaction (PCR technology).

The cloned polynucleotide of the invention may alternatively be termed "DNA construct" or "isolated DNA sequence", and may in particular be a complementary DNA (cDNA).

It is well established that potassium channels may be formed as heteromeric channels, composed of different subunits. Also it has been established that the potassium channel of the invention may form heteromers with other KCNQ's, in particular KCNQ3 and KCNQ4, when co-expressed with these subunits. In addition, potassium channels can associate with non-homologous subunits (β-subunits), e.g. the KCNE1 (also known as minK or IsK), the KCNE2 (also known as the minK-related peptide (MiRP1), the KCNE3 (also known as MiRP2), the KCNE4 (also known as MiRP3), and/or the KCNE5 (also known as KCNE1L) subunit, that co-assemble and functionally modulate these channels or lead to a specific localisation within the cell.

Therefore, in a preferred embodiment, the polynucleotide of the invention is cloned and either expressed by itself or co-expressed with polynucleotides encoding other subunits, in particular a polynucleotide encoding a KCNQ3 channel subunit or a polynucleotide encoding a KCNQ4 channel subunit.

In another aspect of the invention, isolated and purified KCNQ5 antisence oligonucleotides can be made and a method utilised for diminishing the level of expression of KCNQ5 by a cell comprising administering one or more KCNQ5 antisense oligonucleotides. By KCNQ5 antisense oligonucleotides reference is made to oligonucleotides that have a nucleotide sequence that interacts through base pairing with a specific complementary nucleic acid sequence involved in the expression of KCNQ5 such that the expression of KCNQ5 is reduced. Preferably, the nucleic acid sequence involved in the expression of KCNQ5 is a genomic DNA molecule or mRNA molecule that encodes KCNQ5. This genomic DNA molecule can comprise regulatory regions of the *KCNQ5* gene, the pre- or pro- portions of the *KCNQ5* gene, or the coding sequence for mature KCNQ5 protein.

The term "complementary to a nucleotide sequence" in the context of KCNQ5 antisense oligonucleotides and methods therefor means sufficiently complementary to such a sequence as to allow hybridization to that sequence in a cell, i.e. under physiological conditions. The KCNQ5 antisense oligonucleotides preferably comprise a sequence comprising of from about 8 to about 100 nucleotides, more preferably of from about 15 to about 30 nucleotides.

The KCNQ5 antisense oligonucleotides can also include derivatives which comprise a variety of modifications that confer resistance to nucleolytic degradation such as e.g. modified internucleoside linkages modified nucleic acid bases and/or sugars and the like. Examples of such derivatives include backbone modifications such as phosphotriester, phosphorothioate, methylphosphate, phosphoramidate, phosphorodithioate and formacetal as well as morpholino, peptide nucleic acid analogues and dithioate repeating units.

The usefulness of antisense molecules have been described by e.g. *Toulme & Helene*, Gene 1988 **72** 51-58; *Inouye*, Gene 1988 **72** 25-34; *Uhlmann & Peyman*, Chemical Reviews 1990 **90** 543-584; *Robertson*, Nature Biotechnology 1997 **15** 209; and *Gibbons & Dzau*, Science 1996 **272** 689-693, which publications are hereby incorporated by reference.

Biological Sources

The isolated polynucleotide of the invention may be obtained from any suitable human or animal source. In a preferred embodiment, which the polynucleotide of the invention is cloned from, or produced on the basis of a cDNA library, e.g. of the retina, skeletal muscle, or brain, in particular the cerebral cortex, occipital pole, frontal

and temporal lobes, putamen and the hippocampus, and in the piriform cortex, the entorhinal cortex, the pontine medulla and the facial nucleus, and in the cerebellum. In a more preferred embodiment, which the polynucleotide of the invention is cloned from, or produced on the basis of a cDNA library of the thalamus. Commercial cDNA libraries are available from e.g. Stratagene and Clontech.

The KCNQ5 gene of the invention has been localised to the long arm of chromosome 6 (6q14).

The isolated polynucleotide of the invention may be obtained methods known in the art, e.g. those described in the working examples below.

Preferred Polynucleotides

In a preferred embodiment, polynucleotide of the invention has the polynucleotide sequence presented as SEQ ID NO: 1.

In another preferred embodiment the polynucleotide of the invention is a sequence giving rise to KCNQ5 channels subunits comprising one or more substitutions.

In another preferred embodiment the polynucleotide of the invention is a sequence giving rise to KCNQ5 channels subunits comprising one or more substitutions in the conserved regions, as defined in more details below.

It has been demonstrated that KCNQ channels often show alternative splicing and therefore may occur as isoforms originating from the same gene. Such isoforms as well as the different cDNA sequences from which they occurred are also contemplated within the scope of the present invention.

Finally the genes encoding KCNQ channel subunits in other species have been found to differ slightly from the human genes. However, genes of other species, e.g. mouse, rat, monkey, rabbit, etc., are also contemplated within the scope of the present invention.

Recombinantly Produced Polypeptides

In another aspect the invention relates to novel KCNQ5 proteins. More specifically, the invention relates to substantially pure functional polypeptides that have the electrophysiological and pharmacological properties of a KCNQ5 channel, or KCNQ5 channel subunits. The novel polypeptides of the invention may be obtained by the polynucleotides of the invention using standard recombinant DNA technology.

In a preferred embodiment, a polypeptide of the invention is the KCNQ5 potassium channel having the amino acid sequence presented as SEQ ID NO: 2, and biologically active fragments hereof.

Modifications of this primary amino acid sequence may result in proteins which have substantially equivalent activity as compared to the unmodified

counterpart polypeptide, and thus may be considered functional analogous of the parent proteins. Such modifications may be deliberate, e.g. as by site-directed mutagenesis, or they may occur spontaneous, and include splice variants, isoforms, homologues from other species, and polymorphisms. Such functional analogous are also contemplated according to the invention.

Moreover, modifications of this primary amino acid sequence may result in proteins which do not retain the biological activity of the parent protein, including dominant negative forms, etc. A dominant negative protein may interfere with the wild-type protein by binding to, or otherwise sequestering regulating agents, such as upstream or downstream components, that normally interact functionally with the polypeptide. Such dominant negative forms are also contemplated according to the invention.

In the context of this invention, the term "variant polypeptide" means a polypeptide (or protein) having an amino acid sequence that differs from the sequence presented as SEQ ID NO: 2 at one or more amino acid positions. Such variant polypeptides include the modified polypeptides described above, as well as conservative substitutions, splice variants, isoforms, homologues from other species, and polymorphisms.

As defined herein, the term "conservative substitutions" denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative substitutions include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine, and the like. The term conservative substitution also include the use of a substituted amino acid residue in place of an un-substituted parent amino acid residue provided that antibodies raised to the substituted polypeptide also immune-react with the un-substituted polypeptide.

KCNQ1 Numbering System

In the context of this invention, amino acid residues (as well as nucleic acid bases) are specified using the established one-letter symbol.

By aligning the amino acid sequences of a polypeptide of the present invention to those of the known polypeptides, a specific amino acid numbering system may be employed, by which system it is possible to unambiguously allot an amino acid position number to any amino acid residue in any KNCQ channel protein, which amino acid sequence is known.

Such an alignment is presented in Table 1, below. Using the ClustalX computer alignment program [*Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, & Higgins DG*: The ClustalX windows interface: flexible strategies for multiple sequence

alignment aided by quality analysis tools; Nucleic Acids Res. 1997 **25** (24) 4876-82], and the default parameters suggested herein, the amino acid sequence of a polypeptide of the present invention (hKCNQ5) and the amino acid sequences of the known polypeptides hKCNQ2-4 are aligned with, and relative to, the amino acid sequences of the known polypeptide hKCNQ1 (also known as KvLQT1). In the context of this invention this numbering system is designated the KCNQ1 Numbering System.

In describing the various enzyme variants produced or contemplated according to the invention, the following nomenclatures have been adapted for ease of reference:

Original amino acid / Position / Substituted amino acid

According to this nomenclature the substitution of serine for glycine at position 329 of Table 1 is designated as "G329S".

Table 1

CLUSTAL X Multiple Sequence Alignment

KCNQ1 Numbering

```
hKCNQ1 MAAASSPPRA E-----RKRW GWGRLPGARR GSAGLAKKCP FSLELAEGG- ---P--AGGA  60
hKCNQ2 MVQKSR---- ---------- NGGVYPGPSG EKKLKVG--- -FVGLDPG-- -------APD
hKCNQ3 MGLKARRAAG AAGGGGDGGG GGGGAANPAG GDAAAAGDEE RKVGLAPGDV EQVTLALGAG
hKCNQ4 MAEAPPRR-- ---------L GLGPPPGDAP RAELVALT-- -AVQSEQGE- -------AGG
hKCNQ5 MKDVES---- ---------- GRGRVLLNSA AARGDGLLLL GTRAATLGG- -------GGG
       *                     *                     *

hKCNQ1 LYAPIAPGAP GPAPPASPAA PAAPPVASDL GPRPPVSLDP RVSIYSTRRP VLARTHVQGR 120
hKCNQ2 STRDGALLIA G-----SEAP KRGSILSKPR AGGAGAGKPP KRN-AFYRK- ------LQNF
hKCNQ3 ADKDGTLLLE GGG---RDEG QRRTPQGIGL LAKTPLSRPV KRNNAKYRR- ------IQTL
hKCNQ4 GGSPRRLGLL G-----SPLP PGAPLPGPGS GSGSACGQRS SAAHKRYRR- ------LQNW
hKCNQ5 GLRESRRGKQ G---------- ARMSLLGKPL SYTS--SQSC RRN-VKYRR- ------VQNY
                  *                                 *       *

hKCNQ1 VYNFLERPTG WKCFVYHFAV FLIVLVCLIF SVLSTIEQYA ALATGTLFWM EIVLVVFFGT 180
hKCNQ2 LYNVLERPRG W-AFIYHAYV FLLVFSCLVL SVFSTIKEYE KSSEGALYIL EIVTIVVFGV
hKCNQ3 IYDALERPRG W-ALLYHALV FLIVLGCLIL AVLTTFKEYE TVSGDWLLLL ETFAIFIFGA
hKCNQ4 VYNVLERPRG W-AFVYHVFI FLLVFSCLVL SVLSTIQEHQ ELANECLLIL EFVMIVVFGL
hKCNQ5 LYNVLERPRG W-AFIYHAFV FLLVFGCLIL SVFSTIPEHT KLASSCLLIL EFVMIVVFGL
        * **** * *    **    ** * **    * *           *    *     **

                        _____Site 1_____       _____Site 2_____
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```
hKCNQ1 EYVVRLWSAG CRSKYVGLWG RLRFARKPIS IIDLIVVVAS MVVLCVGSKG QVFATSAIRG 240
hKCNQ2 EYFVRIWAAG CCCRYRGWRG RLKFARKPFC VIDIMVLIAS IAVLAAGSQG NVFATSALRS
hKCNQ3 EFALRIWAAG CCCRYKGWRG RLKFARKPLC MLDIFVLIAS VPVVAVGNQG NVLATS-LRS
hKCNQ4 EYIVRVWSAG CCCRYRGWQG RFRFARKPFC VIDFIVFVAS VAVIAAGTQG NIFATSALRS
hKCNQ5 EFIIRIWSAG CCCRYRGWQG RLRFARKPFC VIDTIVLIAS IAVVSAKTQG NIFATSALRS
       *   * * ** *   * *  * *  *****     *  *  **   *      *    ***  * *
                                          _________Site 3_______  _____Site

hKCNQ1 IRFLQILRML HVDRQGGTWR LLGSVVFIHR QELITTLYIG FLGLIFSSYF VYLAEKDAVN 300
hKCNQ2 LRFLQILRMI RMDRRGGTWK LLGSVVYAHS KELVTAWYIG FLCLILASFL VYLAEK----
hKCNQ3 LRFLQILRML RMDRRGGTWK LLGSAICAHS KELITAWYIG FLTLILSSFL VYLVEKDVPE
hKCNQ4 MRFLQILRMV RMDRRGGTWK LLGSVVYAHS KELITAWYIG FLVLIFASFL VYLAEKD---
hKCNQ5 LRFLQILRMV RMDRRGGTWK LLGSVVYAHS KELITAWYIG FLVLIFSSFL VYLVEKD---
         *******    ** ****  ****    *   ** *  *** ** **  *   *** **
       4_________________                 _________Site 5_________

hKCNQ1 -----ESGRV EFGSYADALW WGVVTVTTIG YGDKVPQTWV GKTIASCFSV FAISFFALPA 360
hKCNQ2 ----GE--ND HFDTYADALW WGLITLTTIG YGDKYPQTWN GRLLAATFTL IGVSFFALPA
hKCNQ3 VDAQGEEMKE EFETYADALW WGLITLATIG YGDKTPKTWE GRLIAATFSL IGVSFFALPA
hKCNQ4 -------ANS DFSSYADSLW WGTITLTTIG YGDKTPHTWL GRVLAAGFAL LGISFFALPA
hKCNQ5 -------ANK EFSTYADALW WGTITLTTIG YGDKTPLTWL GRLLSAGFAL LGISFFALPA
                 *  *** ** **  *  *** **** * **  *      *      *******
                          ________P-loop________      ___________Site 6__

hKCNQ1 GILGSGFALK VQQKQRQKHF NRQIPAAASL IQTAWRCYAA E---NPDSST WKIYIRKAP- 420
hKCNQ2 GILGSGFALK VQEQHRQKHF EKRRNPAAGL IQSAWRFYAT NLSRTDLHST WQYYERTVT-
hKCNQ3 GILGSGLALK VQEQHRQKHF EKRRKPAAEL IQAAWRYYAT NPNRIDLVAT WRFYESVVS-
hKCNQ4 GILGSGFALK VQEQHRQKHF EKRRMPAANL IQAAWRLYST DMSRAYLTAT WYYYDSILPS
hKCNQ5 GILGSGFALK VQEQHRQKHF EKRRNPAANL IQCVWRSYAA D-EKSVSIAT WKPHLKALHT
       ****** *** **   *****       ** * **  ** *           * *
       ______

hKCNQ1 -------RSH TLLS------ PSPKPK---- ---------- ---------- -----KSVVV 480
hKCNQ2 -------VPM YRLIPP--LN QLELLRNLKS KSGLAFRK-- -------DPP PEPSPSQKVS
hKCNQ3 -------FPF FRKE------ QLEAAS---- ---------- ---------- ---S--QKLG
hKCNQ4 FRELALLFEH VQRARNGGLR PLEVRRAPVP DGAPSRYPPV ATCHRPGSTS FCPGESSRMG
hKCNQ5 -------CSP TKKE------ QGEASS---- ---------- ---------- -----SQKLS
                  -            Alternatively Spliced            -

hKCNQ1 KKKKFKLDKD NGVTPGEKML TVPH-ITCDP PEERRLDHFS VDGYDSSVRK SPTLLEVS-M 540
hKCNQ2 LKDRV-FSSP RGVAAKGKGS PQAQTVRRSP SADQSLED-- ---SPSKVPK SWSFGDRSRA
hKCNQ3 LLDRVRLSNP RGSNTKGK-- ------LFTP LNVDAIEE-- ---SPSKEPK PVGLNNKERF
hKCNQ4 IKDRIRMGSS QRRTGPSKQQ LAPPTMPTSP SSEQVGEAT- ---SPTKVQK SWSFNDRTRF
hKCNQ5 FKERVRMASP RGQSIKSRQA SVGD--RRSP STDITAEG-- ---SPTKVQK SWSFNDRTRF
                                        *                  *
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```
hKCNQ1 PHFMRTNS-- ----FAEDLD LEGETLLTPI TH-----ISQ LREHHRATIK VIRRMQYFVA 600
hKCNQ2 RQAFRIKGAA S-RQNSEEAS LPGEDIVDDK SCPCEFVTED LTPGLKVSIR AVCVMRFLVS
hKCNQ3 RTAFRMKAYA F-WQSSEDAG T-GDPMAEDR GYGNDFPIED MIPTLKAAIR AVRILQFRLY
hKCNQ4 RASLRLKP-- --RTSAEDAP S--EEVAEEK SYQCELTVDD IMPAVKTVIR SIRILKFLVA
hKCNQ5 RPSLRLKSSQ PKPVIDADTA LGTDDVYDEK GCQCDVSVED LTPPLKTVIR AIRIMKFHVA
          *                                                  *

hKCNQ1 KKKFQQARKP YDVRDVIEQY SQGHLNLMVR IKELQRRLDQ SIGK-PSLFI SVS--EKS-- 660
hKCNQ2 KRKFKESLRP YDVMDVIEQY SAGHLDMLSR IKSLQSRVDQ IVGRGPAITD KDR--TKG--
hKCNQ3 KKKFKETLRP YDVKDVIEQY SAGHLDMLSR IKYLQTRIDM IFTPGPPSTP KHKKSQKGSA
hKCNQ4 KRKFKETLRP YDVKDVIEQY SAGHLDMLGR IKSLQTRVDQ IVGRGPGDRK AREKGDKG--
hKCNQ5 KRKFKETLRP YDVKDVIEQY SAGHLDMLCR IKSLQTRVDQ ILGKGQITSD KKSREKIT--
       * **     * *** ****** * ***    * ** ** *  *
       _________A-domain_____________________________________

hKCNQ1 ---------- ---------K DRG--SNTIG ARLNRVEDKV TQLDQRLALI TDMLHQLLSL 720
hKCNQ2 ---------- -------PAE AELPEDPSMM GFLGKVEKQV LSMEKKLDFL VNIYMQRMGI
hKCNQ3 FTFPSQQSPR NEPYVARPST SEI-EDQSMM GKFVKVERQV QDMGKKLDFL VDMHMQHMER
hKCNQ4 ---------- -------PSD AEVVDEISMM GRVVKVEKQV QSIEHKLDLL LGFYSRCLRS
hKCNQ5 ---------- --------AE HETTDDLSML GRVVKVEKQV QSIESKLDCL LDIYQQVLRK
                                          **  *       *

hKCNQ1 HGGSTPG--- SGGPPREGG- -AHITQPCGS G--GSVDPEL FLPSNTLPTY EQLTVP-RRG 780
hKCNQ2 PPTETE---- AYFGAKEPEP APPYHSPEDS REHVDRHGCI VKIVRSSSST GQKNF----S
hKCNQ3 LQVQVT---- EYYPTKGTSS PAEAEKKEDN R-YSDLKTII CNYSETGPPE PPYSFH-QVT
hKCNQ4 GTSASLGA-- VQVPLFDPDI TSDYHSPVDH E-DISVSAQT LSISRSVSTN MD--------
hKCNQ5 GSASALALAS FQIPPFECEQ TSDYQSPVDS KDLSGSAQNS GCLSRSTSAN ISRGLQFILT

hKCNQ1 PDEGS----- ---------- ---------- ---------- ---------- ---------- 840
hKCNQ2 APPAAPPVQC PPSTSWQPQS HPRQGH---- ---------- ---GTSPVGD HGSLVRIPPP
hKCNQ3 IDKVSPYGFF AHDPVNLPRG GPSSGKV--- ---------- ---QATPPSS ATTYVERPTV
hKCNQ4 ---------- ---------- ---------- ---------- ---------- ----------
hKCNQ5 PNEFSAQTFY ALSPTMHSQA TQVPISQSDG SAVAATNTIA NQINTAPKPA APTTLQIPPP

hKCNQ1 ---------- ---------- ---------- ---------- ---------- ---------- 900
hKCNQ2 PAHERSLSAY GGGN-RASME FLRQEDTPGC R-PPEGTLRD SDTSISIPSV DHEELERSFS
hKCNQ3 LPILTLLDSR VSCH-SQADL QGPYSDRISP R-QRRSITRD SDTPLSLMSV NHEELERSPS
hKCNQ4 ---------- ---------- ---------- ---------- ---------- ----------
hKCNQ5 LPAIKHLPRP ETLHPNPAGL QESISDVTTC LVASKENVQV AQSNLTKDRS MRKSFDMGGE
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hKCNQ1 ---------- ---------- ---------- ---------- ---------- ---------- 960
hKCNQ2 GFSISQSKEN LDALNSCYAA VAPCAKVRPY IAEGESDTD- ----SDLCTP CGPPPRSATG
hKCNQ3 GFSISQDRDD YVFGPNGGSS WM---REKRY LAEGETDTD- ----TDPFTP SGSMPLSSTG
hKCNQ4 ---------- ---------- ---------- ---------- ---------- ----------
hKCNQ5 TLLSVCPMVP KDLGKSLSVQ NLIRSTEELN IQLSGSESSG SRGSQDFYPK WRESKLFITD

hKCNQ1 ---------- ------                                              976
hKCNQ2 EGPFGDVGWA GPRK--
hKCNQ3 DG-ISDSVWT PSNKPI
hKCNQ4 ---------- ------
hKCNQ5 EEVGPEETET DTFARI
```

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hKCNQ1: Human KCNQ1 [*Wang, Q et al.*; Nature Genet. 1996 **12** 17-23]
hKCNQ2: Human KCNQ2 [*Biervert et al.*; Science 1998 **279** 403-406]
hKCNQ3: Human KCNQ3 [*Schroeder et al.*; Nature 1998 **396** 687-690]
hKCNQ4: Human KCNQ4 [*Kubisch et al.*; Cell 1999 **96** (3) 437-46]
hKCNQ5: Human KCNQ5; A protein of the invention
\- No amino acid in this position.
* Indicates positions which holds a single, fully conserved residue (Conserved regions).

Preferred variants are the splice variants at positions 432-476 (KCNQ1 Numbering) holding the following amino acid residues:

1) KKE------ QGEASS---- ------NKFC SNKQKLFRMY TSRKQS;
2) KKE------ QGEASS---- ---------- ---------- ------;
3) --------- ---------- ---------- ---------- ------; or
4) --------- ---------- ------NKFC SNKQKLFRMY TSRKQS.

Another preferred variants is G329S (KCNQ1 numbering), or KCNQ5/G278S ("KCNQ5 numbering").

Biological Activity

Ion channels are excellent targets for drugs. The polynucleotide of the invention encodes a potassium channel, which has been termed KNCQ5.

KCNQ5, or heteromeric channels containing the KCNQ5 subunit, may be a particularly interesting target for the treatment of diseases or adverse conditions of the CNS, including affective disorders, Alzheimer's disease, anxiety, ataxia, CNS damage caused by trauma, stroke or neurodegenerative illness, cognitive deficits, compulsive behaviour, dementia, depression, Huntington's disease, learning deficiencies, mania, memory impairment, memory disorders, memory dysfunction, motion disorders, motor

disorders, motor neuron doseases, myokymia, neurodegenerative diseases, Parkinson's disease and Parkinson-like motor disorders, phobias, Pick's disease, psychosis, schizophrenia, seizures, incl. epileptic seizures, spinal cord damage, stroke, tremor, seizures, convulsions and epilepsy.

The novel polynucleotides of the invention may in itself be used as a therapeutic or diagnostic agent. For gene therapy, the person skilled in the art may use sence or antisense nucleic acid molecules as therapeutic agents for KCNQ-related indications.

Heteromers Formed by KCNQ Subunits

The KCNQ channels described so far function physiologically as heteromers. KCNQ1 associates with KCNE1 (also known as minK or IsK); KCNQ2 and KCNQ3 form heteromeric channels that underlie the M-current, an important determinant of neuronal excitability that is regulated by several neurotransmitters, and KCNQ4 is supposed to combine with KCNQ3 to mediate the I_M-like current in the outer hair cells.

Like other KCNQ channel subunits, KCNQ5 may interact with other subunits, e.g. KCNE1 or other KCNQ channel subunits, and in particular with KCNQ3, and with KCNQ4. Currents from homomeric KCNQ3 are very small and often cannot be distinguished from *Xenopus* oocyte background currents. Co-expression of KCNQ3 with KCNQ5 markedly increased current amplitudes. Co-expression of KCNQ4 with KCNQ5 markedly decreased current amplitudes.

Antibodies

The polypeptides of the invention can be used to produce antibodies which are immunoreactive or bind to epitopes of these polypeptides. Polyclonal antibodies which consist essentially of pooled monoclonal antibodies with different specificities, as well as distinct monoclonal antibody preparations may be provided. Polyclonal antibodies which are made up of pooled monoclonal antibodies with different specificities, as well as distinct monoclonal antibody preparations may be provided.

The preparation of polyclonal and monoclonal antibodies is well known in the art. Polyclonal antibodies may in particular be obtained as described by e.g. *Green et al.*: "Production of Polyclonal Antisera" in Immunochemical Protocols (*Manson*, Ed.); Humana Press, 1992, Pages 1-5; *Coligan et al.*: "Production of Polyclonal Antisera in rabbits, rats, Mice and Hamsters" in Current Protocols in Immunology, 1992, Section 2.4.1; and *Ed Harlow and David Lane* (Eds.) in "Antibodies; A laboratory manual", Cold Spring Harbor Lab. Press 1988; which protocols are hereby incorporated by reference.

Monoclonal antibodies may in particular be obtained as described by e.g. *Kohler & Milstein*, Nature 1975 **256** 495; Coligan et al. in Current Protocols in Immunology, 1992, Sections 2.5.1 - 2.6.7; *Harlow et al.* in Antibodies: A Laboratory Manual; Cold Spring Harbor Pub., 1988, Page 726; which protocols are hereby incorporated by reference.

Briefly, monoclonal antibodies may be obtained by injecting e.g. mice with a composition comprising an antigen, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones that produce the antibodies to the antigen, and isolating the antibodies from the hybridoma cultures.

Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques, including affinity chromatography with protein A Sepharose, size-exclusion chromatography, and ion-exchange chromatography, see. e.g. *Coligan et al.* in Current Protocols in Immunology, 1992, Sections 2.7.1 - 2.7.12, and Sections 2.9.1 - 2.9.3; and *Barnes et al.*: "Purification of Immunoglobulin G (IgG)" in Methods in Molecular Biology; Humana Press, 1992, Vol. 10, Pages 79-104.

The polyclonal or monoclonal antibodies may optionally be further purified, e.g. by binding to and elution from a matrix to which the polypeptide, to which the antibodies were raised, is bound.

Antibodies which bind to the polypeptide of the invention can be prepared using an intact polypeptide or fragments containing small peptides of interest as the immunising antigen. The polypeptide used to immunise an animal may be obtained by recombinant DNA techniques or by chemical synthesis, and may optionally be conjugated to a carrier protein. Commonly used carrier proteins which are chemically coupled to the peptide include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid. The coupled peptide may then be used to immunise the animal, which may in particular be a mouse, a rat, a hamster or a rabbit.

Genetically Manipulated Cells

In a third aspect the invention provides a cell genetically manipulated by the incorporation of the heterologous polynucleotide of the invention. The cell of the invention may in particular be genetically manipulated to transiently or stably express, over-express or co-express a KCNQ5 channel subunit as defined above. Methods of transient and stable transfer are known in the art.

The polynucleotide of the invention may be inserted into an expression vector, e.g. a plasmid, virus or other expression vehicle, and operatively linked to

expression control sequences by ligation in a way that expression of the coding sequence is achieved under conditions compatible with the expression control sequences. Suitable expression control sequences include promoters, enhancers, transcription terminators, start codons, splicing signals for introns, and stop codons, all maintained in the correct reading frame of the polynucleotide of the invention so as to permit proper translation of mRNA. Expression control sequences may also include additional components such as leader sequences and fusion partner sequences.

The promoter may in particular be a constitutive or an inducible promoter. When cloning in bacterial systems, inducible promoters such as pL of bacteriophage γ, plac, ptrp, ptac (ptrp-lac hybrid promoter), may be used. When cloning in mammalian systems, promoters derived from the genome of mammalian cells, e.g. the TK promoter or the metallothionein promoter, or from mammalian viruses, e.g. the retrovirus long terminal repeat, the adenovirus late promoter or the vaccinia virus 7.5K promoter, may be used. Promoters obtained by recombinant DNA or synthetic techniques may also be used to provide for transcription of the polynucleotide of the invention.

Suitable expression vectors typically comprise an origin of expression, a promoter as well as specific genes which allow for phenotypic selection of the transformed cells, and include vectors like the T7-based expression vector for expression in bacteria [*Rosenberg et al*; Gene 1987 **56** 125], the pMSXND expression vector for expression in mammalian cells [*Lee and Nathans*, J. Biol. Chem. 1988 **263** 3521], baculovirus derived vectors for expression in insect cells, and the oocyte expression vector PTLN [*Lorenz C, Pusch M & Jentsch T J*: Heteromultimeric CLC chloride channels with novel properties; Proc. Natl. Acad. Sci. USA 1996 **93** 13362-13366].

In a preferred embodiment, the cell of the invention is an eukaryotic cell, in particular a mammalian cell, an oocyte, or a yeast cell. In a more preferred embodiment, the cell of the invention is a human embryonic kidney (HEK) cell, a HEK 293 cell, a BHK21 cell, a Chinese hamster ovary (CHO) cell, a *Xenopus laevis* oocyte (XLO) cell, a COS cell, or any other cell line able to express KCNQ potassium channels.

When the cell of the invention is an eukaryotic cell, incorporation of the heterologous polynucleotide of the invention may be in particular be carried out by infection (employing a virus vector), by transfection (employing a plasmid vector), or by calcium phosphate precipitation, microinjection, electroporation, lipofection, or other physical-chemical methods known in the art.

In a further preferred embodiment, the cell of the invention is genetically manipulated to co-express KCNQ5 and KCNQ1 channel subunits; KCNQ5 and KCNQ2 channel subunits; KCNQ5 and KCNQ3 channel subunits; KCNQ5 and

KCNQ4 channel subunits; KCNQ5 and KCNQ1 and KCNQ2 channel subunits; KCNQ5 and KCNQ1 and KCNQ3 channel subunits; KCNQ5 and KCNQ2 and KCNQ3 channel subunits; KCNQ5 and KCNQ1 and KCNQ4 channel subunits; KCNQ5 and KCNQ2 and KCNQ4 channel subunits; KCNQ5 and KCNQ3 and KCNQ4 channel subunits; KCNQ5 and KCNQ1 and KCNQ2 and KCNQ3 channel subunits; KCNQ5 and KCNQ1 and KCNQ2 and KCNQ4 channel subunits; KCNQ5 and KCNQ1 and KCNQ3 and KCNQ4 channel subunits, or KCNQ5 and KCNQ2 and KCNQ3 and KCNQ4 channel subunits.

In another preferred embodiment, membrane preparations are provided. The membrane preparations of the invention may typically be used for screening purposes, and may be obtained by standard techniques.

In a preferred embodiment, frozen intact cells of the invention are homogenised while in cold water suspension and a membrane pellet is collected after centrifugation. The pellet may then be washed in cold water and dialysed to remove endogenous ligands that could compete for binding in the assays. The dialysed membranes may be used as such, or after storage in lyophilised form.

KCNQ5 Active Chemical Compounds

In another aspect the invention relates to chemical compounds capable of binding to, and showing activity at potassium channels containing one or more KCNQ5 subunits. In the context of this invention such compounds are termed KCNQ5 active compounds.

The KCNQ5 active compounds of the invention have therapeutic potential, and may be used for the manufacture of pharmaceutical compositions.

The KCNQ5 active compounds of the invention may in particular be used in diagnosis, treatment, prevention or alleviation of diseases related to diseases or adverse conditions of the CNS, including affective disorders, Alzheimer's disease, anxiety, ataxia, CNS damage caused by trauma, stroke or neurodegenerative illness, cognitive deficits, compulsive behaviour, dementia, depression, Huntington's disease, mania, memory impairment, memory disorders, memory dysfunction, motion disorders, motor disorders, neurodegenerative diseases, Parkinson's disease and Parkinson-like motor disorders, phobias, Pick's disease, psychosis, schizophrenia, spinal cord damage, stroke, tremor, seizures, convulsions and epilepsy.

Currently two compounds have been identified. As a preferred embodiment the invention therefore provides 1,3-dihydro-1-phenyl-3,3-bis-(4-pyridylmethyl)-2H-indol-2-one (Linopirdine) and 10,10-bis-(4-pyridinyl-methyl)-9-(10H)-antracenone (XE991) for use in the manufacture of a pharmaceutical composition for the diagnosis, treatment, prevention or alleviation of the above diseases.

Screening of Drugs

In a further aspect the invention provides methods for screening for KCNQ5 active compounds, i.e. chemical compounds capable of binding to, and showing activity at potassium channels containing one or more KCNQ5 subunits. The activity determined may be inhibitory activity, stimulating activity, or other modulatory activity. In particular the KCNQ5 active compound may induce a second messenger response, which cause a change of the molecular characteristics of the cell, e.g. the ion flux, enzyme activation, changes in the level of intracellular Ca^{2+} or H^{+}, changes cyclic nucleotides such as cAMP, cADP, cGMP, and cGDP, etc.

Therefore, in another aspect, the invention provides a method for identifying functional ligands for a human potassium channel, comprising a KCNQ5 subunit, which method comprises transfecting cells with one or more polypeptides of the invention, encoding a KCNQ5 channel subunit, and detecting the effect on the signal transduction pathway caused in these cells by binding of the ligands to the receptor by a reporter system.

Such chemical compounds can be identified by one of, or both methods described below.

Binding Studies

Binding studies are usually carried out by subjecting the target to binding with a labelled, selective agonist (binding agent), to form a labelled complex, followed by determination of the degree of displacement caused by the test compound upon addition to the complex.

In a specific aspect the invention provides a method of screening a chemical compound for capability of binding to a potassium channel comprising at least one KCNQ5 channel subunit, which method comprises the steps of (i) subjecting a KCNQ5 channel subunit containing cell to the action of a KCNQ5 binding agent to form a complex with the KCNQ5 channel subunit containing cell; (ii) subjecting the complex of step (i) to the action of the chemical compound to be tested; and (iii) detecting the displacement of the KCNQ5 binding agent from the complex with the KCNQ5 channel subunit containing cell.

The KCNQ5 channel subunit containing cell preferably is a cell of the invention as described above.

The KCNQ5 binding agent preferably is a radioactively labelled 1,3-dihydro-1-phenyl-3,3-bis-(4-pyridylmethyl)-2H-indol-2-one (Linopirdine); or 10,10-bis-(4-pyridinyl-methyl)-9-(10H)-antracenone (XE991).

In a even more preferred embodiment, the binding agent is labelled with ^{3}H, and the displacement of the KCNQ5 binding agent from the complex with the

KCNQ5 channel subunit containing cell is detected by measuring the amount of radioactivity by conventional liquid scintillation counting.

Activity Studies

The KCNQ5 channel agonists may affect the potassium channel in various ways. The agonist may in particular show inhibitory activity, stimulating activity, or other modulatory activity.

In a specific aspect the invention provides a method for determining the activity at potassium channels containing one or more KCNQ5 subunits. According to this method a KCNQ5 channel subunit containing cell is subjecting to the action of the chemical compound to be tested, and the activity is detected by way of monitoring the membrane potential, the current, the potassium flux, or the secondary calcium influx of the KCNQ5 channel subunit containing cell, preferably a genetically manipulated as described above.

The membrane potential and the current may be monitored by electrophysiologic methods, including patch clamp techniques, such as current clamp technology and two-electrode voltage clamp technology, or by spectroscopic methods, such as fluorescence methods.

In a preferred embodiment, monitoring of the membrane potential of the KCNQ5 channel subunit containing cell is performed by patch clamp techniques.

In another preferred embodiment, monitoring of the membrane potential of the KCNQ5 channel subunit containing cell is performed by spectroscopic methods, e.g. using fluorescence methods. In a more specific embodiment, the KCNQ5 channel subunit containing cell is mixed with a membrane potential indicating agent, that allow for a determination of changes in the membrane potential of the cell, caused by the addition of the test compound. The membrane potential indicating agent may in particular be a fluorescent indicator, preferably $DIBAC_4(3)$, DiOC5(3), and DiOC2(3).

In yet a preferred embodiment, monitoring of the membrane potential of the KCNQ5 channel subunit containing cell is performed by spectroscopic methods, e.g. using a FLIPR assay (Fluorescence Image Plate Reader; available from Molecular Devices).

Screening of Genetic Material

In a further aspect the invention relates to the use of a polynucleotide sequence of the invention for the screening of genetic materials. By this method, individuals bearing a gene identical or homologous to a polynucleotide of the invention may be identified.

In the screening method of the invention, a polynucleotide of the invention, or any fragment or sub-sequence hereof, is employed. For the identification of

individuals bearing mutated genes preferably the mutated forms of the polynucleotide represented by SEQ ID NO: 1 are employed.

In the screening method of the invention only short sequences needs to be employed depending on the actual method used. For SSCA, several hundreds of base pairs may be needed, for oligonucleotide or PCR hybridisation only of from about 10 to about 50 basepairs may be needed.

The screening may be accomplished by conventional methods, including hybridisation, SSCA analysis, and microarray technology (DNA chip technology). The hybridisation protocol described above represents a suitable protocol for use in a screening method of the invention.

Transgenic Animals

Transgenic animal models provide the means, *in vivo*, to screen for therapeutic compounds. Since KCNQ5 is expressed also in brain, they may be helpful in screening for drugs effective in CNS disorders, e.g. epilepsy.

By transgene is meant any piece of polynucleotide which is inserted by artifice into a cell, and thus becomes part of the genome of the organism that develops from that cell. Such a transgene may include a gene which is partly or entirely heterologous (i.e. foreign) to the transgenic organism, or it may represent a gene homologous to an endogenous gene of the organism.

By a transgenic animal is meant any organism holding a cell which includes a polynucleotide sequence which is inserted into that cell by artifice, and which cell becomes part of the transgenic organism which develops from that cell. Such a transgene may be partly or entirely heterologous to the transgenic animal. Although transgenic mice represent a preferred embodiment of the invention, other transgenic mammals including, but not limited to transgenic rodents (e.g. hamsters, guinea pigs, rabbits and rats), and transgenic pigs, cattle, sheep and goats may be created by standard techniques and are included in the invention.

Preferably, the transgene is inserted by artifice into the nuclear genome.

Knock-out and Knock-in Animals

The transgenic knock-out animal models may be developed by homologous recombination of embryonic stem cells with constructs containing genomic sequence from the *KCNQ5* gene, that lead to a loss of function of the gene after insertion into the endogenous gene.

By knock-out mutation is meant an alteration in the polynucleotide sequence that reduces the biological activity of the polypeptide normally encoded therefrom. In order to create a true knock-out model, the biological activity of the expressed polypeptide should be reduced by at least 80% relative to the un-mutated

gene. The mutation may in particular be a substitution, an insertion, a deletion, a frameshift mutation, or a mis-sense mutation. Preferably the mutation is a substitution, an insertion or a deletion.

To further assess the role of KCNQ5 at an organism level, the generation of an animal, preferably a mouse, lacking the intact *KCNQ5* gene, or bearing a mutated *KCNQ5* gene, is desired.

A replacement-type targeting vector, which may be used to create a knock-out model, may be constructed using an isogenic genomic clone, e.g. from a mouse strain such as 129/Sv (Stratagene Inc., La Jolla, CA). The targeting vector may be introduced into a suitably-derived line of embryonic stem (ES) cells by electroporation to generate ES cell lines that carry a profoundly truncated form of the *KCNQ5* gene. The targeted cell lines may then be injected into a mouse blastula stage embryo to generate chimeric founder mice. Heterozygous offspring may be interbred to homozygosity.

Animal models for over-expression may be generated by integrating one or more polynucleotide sequence of the invention into the genome according to standard techniques.

The procedures disclosed herein involving the molecular manipulation of nucleic acids are known to those skilled in the art, and are described by e.g. *Fredrick MA et al.* [*Fredrick MA et al.*: Short Protocols in Molecular Biology; John Wiley and Sons, 1995] and *Sambrook et al.* [*Sambrook et al.*: Molecular Cloning: A Laboratory Manual; 2. Ed., Cold Spring Harbor Lab.; Cold Spring Harbor, NY 1989], and in *Alexandra LJ* (Ed.): Gene Targeting: A practical approach; Oxford University Press (Oxford, New York, Tokyo), 1993.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is further illustrated by reference to the accompanying drawing, in which:

Fig. 1 shows the electrophysiological properties of KCNQ5 currents [I/μA vs. time/seconds]. Two-electrode voltage-clamp current traces from a *Xenopus* oocyte injected with KCNQ5 cRNA. Starting from a holding potential of -80 mV, cells were clamped for 2 seconds to voltages between -80 and +40 mV, in +10 mV steps, followed by a constant test pulse to -30 mV; and

Fig. 2 shows the electrophysiological properties of currents arising from the co-expression of KCNQ5 with KCNQ3 [I/μA vs. time/seconds]. Starting from a holding potential of -80 mV cells were clamped for 2 seconds to voltages between -80 and +40 mV, in +10 mV steps, followed by a constant test pulse to -30 mV; and

Fig. 3 shows the electrophysiological properties of currents arising from the co-expression of KCNQ5 with KCNQ4 (2B) [I/μA vs. time/seconds]. Starting from a holding potential of -80 mV cells were clamped for 2 seconds to voltages between -80 and +40 mV, in +10 mV steps, followed by a constant test pulse to -30 mV;

Figs. 4A-G show the electrophysiological properties of KCNQ5. Both the splice variant I (found in brain) and splice variant III (found in muscle) were expressed in *Xenopus* oocytes and examined by two-electrode voltage clamping. Both variants activate slowly upon depolarisation, but form I (4A) initially activates slower than the muscle form III (4B). Starting from a holding potential of –80 mV, the voltage was stepped for 0.8 seconds to values between –100 and + 40 mV in steps of 10 mV, followed by a voltage step to –30 mV (see inset, panel A). Channel activation by depolarization was fitted (for 2 sec. steps) by a sum of three exponential functions. For a step to +20 mV, the rate constants were $\hat{o}_1$ = 37. 2 ± 2.2 ms, $\hat{o}_2$ = 246 ± 17 ms, and $\hat{o}_3$ = 1112 ± 91 ms for splice variant I, while these constants were $\hat{o}_1$ = 24.5 ± 0.8 ms, $\hat{o}_2$ = 163 ± 6 ms, and $\hat{o}_3$ = 1690 ± 46 ms (± SEM, n=16). This difference in kinetics also results in different curves when a typical M-current protocol is used (4C, variant I; 4D, variant III). Variant I induces currents that kinetically resemble M-currents. In this protocol, the membrane voltage is clamped for 1 second to voltages between –30 and –90 mV in steps of -10 mV, from a holding potential of –30 mV. This was followed by a step to –30 mV (panel C, inset). (4E, 4F), apparent open probabilities of variants I (4E) and variant III (4F) as a function of voltage obtained from tail current analysis as described in Methods. Mean values obtained from 12 oocytes are shown. Fitting a Boltzmann equation yielded $V_{½}$ = -46 ± 1 mV and an apparent gating charge of z = 2.8 ± 0.1 for isoform I, and $V_{½}$ = -48 ± 1 mV and an apparent gating charge of z = 2.5 ± 0.1 for isoform III. For this fit, values at potentials more positive than +10 mV were excluded, as these are probably affected by a second (inactivation) gating process which leads to a decrease of apparent p_{open} at more positive potentials. (4G), ion selectivity of KCNQ5 currents (variant I). Extracellular sodium was replaced by equimolar amounts of potassium. The reversal potential is shown as a function of the potassium concentration. This yielded a slope of 51 mV/decade potassium concentration, indicating a highly selective potassium channel. Data are from 10 oocytes from 2 different batches;

Figs. 5A-C show the pharmacology of KCNQ5 and KCNQ3/5 heteromers. (5A) Inhibition of KCNQ5 homomers by extracellular linopirdine (*down triangles*), XE991 (*up triangles*) and TEA (*squares*). IC_{50} values of 51 ± 5 μM, 65 ± 4 μM and 71 ± 17 mM, respectively, were obtained from the plotted fit curves. (5B) Niflumic acid alters the voltage dependence of the apparent p_{open}. In the presence of 500 μM niflumic acid (*open circles*), the voltage dependence is shifted about 20 mV towards negative potentials. (5C) TEA sensitivity is altered in KCNQ3/5 heteromers.

Coexpression of KCNQ5 and KCNQ3 (1:1) (*diamonds*) increased the IC_{50} value to ~ 200 mM, coexpression with the KCNQ3 (T323Y) mutant (*circles*) decreased the IC_{50} to ~ 30 mM. Data points in panels A and C are the means ± SEM of 4 to 12 individual measurements. p_{open} was determined as in Fig. 3;

Fig. 6 shows the inhibition of KCNQ5 currents by stimulating M1 receptors which were co-expressed in *Xenopus* oocytes:

(6A) currents before stimulating the M1 receptor;

(6B) current observed 3 minutes after applying 10 µM muscarine. The pulse protocol is shown in the inset of panel b. No effect of 10 µM muscarine or oxotremorine methiodide was found in oocytes injected with KCNQ5 alone; and

Figs. 7A-C show the interactions between KCNQ2 and KCNQ5 (7A, 7B) and KCNQ3 and KCNQ5 (7C):

(7A) currents at the end of a two second pulse to 0 mV from a holding potential of –80 mV of oocytes injected with different combinations of KCNQ cRNAs (always 10 ng total amount of RNA). The current amplitude elicited by co-injecting KCNQ2 and KCNQ5 could be explained by a linear superposition of currents. Co-injection of KCNQ5 with the dominant negative mutant KCNQ2(G279S) or of KCNQ2 with the equivalent mutant KCNQ5(G278S) lead to a roughly 50% reduction in current amplitude, which is consistent with a lack of interaction since only 50% of WT cRNA was injected;

(7B) normalized current traces of experiments used for panel (A). Currents elicited by the depolarizing pulse to 0 mV are shown. KCNQ2 (labeled Q2) activates faster than KCNQ5 (Q5), and the co-expression of both yielded currents that may be explained by a linear superposition. Co-injecting KCNQ2 with the dominant negative (and otherwise non-functional) mutant KCNQ5(G278S) yielded currents that were kinetically similar to KCNQ2, and currents from a KCNQ5/KCNQ2(G279S) co-injection resembled KCNQ5 currents;

(7C), interactions between KCNQ3 and KCNQ5 measured as in panel (a). KCNQ3 yields only very small currents in Xenopus oocytes. Currents were enlarged when KCNQ5 was co-expressed with small amounts of KCNQ3, and reduced when co-expressed with larger amounts. The dominant negative mutant KCNQ3(G318S) decreased currents significantly below 50% of WT KCNQ5 currents that would be expected from the injected 50% of WT KCNQ5 cRNA in this experiment.

EXAMPLES

The invention is further illustrated with reference to the following examples which are not intended to be in any way limiting to the scope of the invention as claimed.

Example 1

Cloning and Characterisation of KCNQ5 cDNA

Using a near full-length KCNQ3 potassium channel cDNA as a probe, a human thalamus cDNA λGT11 phage library (Clontech, #HL5009b) was screened, and a partial cDNA clone encoding a protein fragment homologous to KCNQ potassium channels was isolated. It was distinct from the known members KCNQ1 (KvLQT1), KCNQ2, KCNQ3 and KCNQ4. We named the novel gene *KCNQ5*. Overlapping cDNA's containing the entire open reading frame were obtained by re-screening the cDNA library and by extending the 5' end in RACE (rapid amplification of cDNA ends) experiments using a Marathon kit (Clontech) with human brain cDNA. A complete cDNA was assembled and cloned into the oocyte expression vector PTLN [*Lorenz C, Pusch M & Jentsch T J*: Heteromultimeric CLC chloride channels with novel properties; Proc. Natl. Acad. Sci. USA 1996 **93** 13362-13366].

The cDNA encodes a polypeptide of 897 amino acids with a predicted mass of 99 kDa (SEQ ID NO: 2). Its overall amino-acid identity to KCNQ1, KCNQ2, KCNQ3 and KCNQ4 is 43%, 58%, 54%, and 61% respectively. Together with these proteins, it forms a distinct branch of the superfamily of voltage-gated potassium channels. As a typical member of this gene family, KCNQ5 has 6 predicted transmembrane domains and a P-loop between transmembrane domains S5 and S6. In potassium channels, which are tetramers of identical or homologous subunits, four of these highly conserved P-loops combine to form the ion-selective pore. As other KCNQ channels, KCNQ5 has a long predicted cytoplasmic carboxy terminus that accounts for about half of the protein. A conserved region present in the carboxy termini of KCNQ1, -2, -3 and -4 is also present in KCNQ5.

The sequence of KCNQ5 predicts several potential sites for phosphorylation by protein kinase C and one for protein kinase A. In contrast to KCNQ1 and KCNQ2, however, it lacks an amino terminal consensus site for cAMP-dependent phosphorylation.

A human multiple tissue Northern blot (Clontech, #7760-1) was probed with a cDNA fragment of KCNQ5. The fragment was labelled with ^{32}P using the Rediprime labelling kit (Amersham). Hybridisation was performed in ExpressHyb solution according to the instructions of the manufacturer (Clontech). The filter was then exposed to Kodak BioMax film for 4 days.

Northern analysis of *KCNQ5* expression in human tissues revealed a band of ≈9 kb in brain.

Example 2

Functional expression of KCNQ5 potassium channel subunits

KCNQ5 was expressed in *Xenopus* oocytes and its activity was investigated by two-electrode voltage clamping.

After linearization of the *KCNQ5*-containing pTLN vector with HpaI, capped cRNA was transcribed *in vitro* using the mMessage mMachine cRNA synthesis kit (Ambion). Usually 5 – 15 ng of cRNA were injected into *Xenopus* oocytes previously isolated by manual defolliculation and short collagenase treatment. In co-expression experiments cRNAs were injected at a 1:1 ratio. Oocytes were kept at 17°C in modified Barth's solution (90 mM NaCl, 1 mM KCl, 0.41 mM $CaCl_2$, 0.33 mM $Ca(NO_3)_2$, 0.82 mM $MgSO_4$, 10 mM HEPES, 100 U penicillin–100 µg streptomycin/ml, pH 7.6).

Standard two-electrode voltage-clamp measurements were performed at room temperature 2-4 days after injection using a Turbotec 05 amplifier (npi instruments, Tamm, Germany) and pClamp 5.5 software (Axon Instruments). Currents were usually recorded in ND98 solution (see Table 2). Linopirdine (RBI, Natick, MA) was prepared as a 100 mM stock solution in DMSO and added to a final concentration of 200 µM to ND98.

Table 2

Solution contents (Concentrations in mM)

ND98	ND 100	KD100	Rb100	Cs100
98 NaCl	100 NaCl	100 KCl	100 RbCl	100 CsCl
2 KCl				
0.2 $CaCl_2$	0.2 $CaCl_2$	0.2 $CaCl_2$	0.2 $CaCl_2$	0.2 $CaCl_2$
2.8 $MgCl_2$	2.8 $MgCl_2$	2.8 $MgCl_2$	2.8 $MgCl_2$	2.8 $MgCl_2$
5 mM HEPES, pH 7.4				

To determine the voltage dependence of apparent open probability, oocytes were clamped for 2 seconds to values between -80 mV to +40 mV, in 10 mV steps, followed by a constant -30 mV test pulse. Tail currents extrapolated to t=0 were obtained from a mono-exponential fit, normalised to the value at 0 mV and used for the analysis of apparent p_{open}. Data analysis used PClamp6 and Microcal Origin 5.0.

Similar to KCNQ1, KCNQ2, KCNQ3, and KCNQ4 also KCNQ5 yielded currents that activated upon depolarisation (Fig. 1). Compared to KCNQ1, KCNQ2

and KCNQ2/3 channels, however, current activation was slower and occurred with a time constant in the order of 600 ms at +20mV (KCNQ2/KCNQ3 channels have a corresponding time constant of ≈300 ms). Deactivation of currents at physiological resting potentials (≈-30mV) was considerably faster (Fig. 1). Similar to KCNQ2, macroscopic currents often showed some inward rectification at positive potentials. KCNQ5 currents were inhibited by more than 80% by 5 mM Ba^{++}.

KCNQ1 assembles with minK (also known as KCNE1 or IsK) to form channels that yield larger currents and activate much slower. We therefore tested by co-expression whether minK affects KCNQ5 as well. At concentrations (1ng minK cRNA per oocyte) leading to drastic changes in KCNQ1 currents in parallel experiments, there was just a slight change in KCNQ5 currents.

Different KCNQ subunits can form heteromeric channels. Co-expression of KCNQ2 with KCNQ3, but not with KCNQ1, gave currents that were about tenfold larger than those from homomeric channels. Since also KCNQ2, KCNQ3 and KCNQ4 are expressed in the brain, we investigated whether these proteins interact functionally. Oocytes co-injected (at the same total cRNA concentration) with KCNQ2 and KCNQ5 cRNAs yielded currents that seemed not different from a linear superposition of currents from the respective homomeric channels.

By contrast, co-expression of KCNQ3 with KCNQ5 yielded currents that were significantly larger than could be explained by a superposition of currents from the respective homomeric channels (Figs. 2A). Further, co-expression of KCNQ5 and KCNQ4 decreased currents when compared to homomeric channels (Fig. 2B).

Linopirdine, a potent and rather specific inhibitor for M-currents, nearly completely inhibits KCNQ2/KCNQ3 channels at a concentration of 200 µM.

This concentration of Linopirdine inhibited KCNQ5 by about 80%.

CLAIMS:

1. An isolated nucleic acid molecule encoding a polypeptide comprising all or a portion of a human, rat or murine KCNQ5 protein.

2. An isolated polynucleotide having a nucleic acid sequence which is capable of hybridising under at least medium stringency conditions with the polynucleotide sequence presented as SEQ ID NO: 1, its complementary strand, or a sub-sequence thereof.

3. The isolated polynucleotide according to claim 2, being at least 65% homologous, preferably more than 70%, more preferred more than 80%, even more preferred more than 90%, most preferred more than 95%, homologous to the polynucleotide sequence presented as SEQ ID NO: 1.

4. The isolated polynucleotide according to any of claims 1-3 being a cloned polynucleotide.

5. The isolated polynucleotide according to claim 4, in which the polynucleotide is cloned from, or produced on the basis of a cDNA library.

6. The isolated polynucleotide according to any of claims 1-5, having the polynucleotide sequence presented as SEQ ID NO: 1.

7. The isolated polynucleotide according to any of claims 1-6, encoding a potassium channel, or a potassium channel subunit.

8. The isolated polynucleotide according to claim 7, encoding the KCNQ5 potassium channel subunit having the amino acid sequence represented by SEQ ID NO: 2.

9. The isolated polynucleotide according to claim 7, encoding a KCNQ5 variant, which variant has an amino acid sequence that has been changed by deletion of an amino acid residue, by insertion of an additional amino acid residue, or by substitution of an amino acid residue at one or more positions.

10. The isolated polynucleotide according to claim 9, which variant has an amino acid sequence that has been changed at one or more positions located in the conserved regions, as defined by Table 1.

11. The isolated polynucleotide according to claim 9, which variant is G329S (KCNQ1 numbering), or KCNQ5/G278S.

12. A vector construct comprising the polynucleotide according to any of claims 1-11.

13. A recombinantly produced polypeptide encoded by the polynucleotide according to claims 1-11.

14. The polypeptide according to claim 13, being a KCNQ5 potassium channel subunit having the amino acid sequence presented as SEQ ID No. 2.

15. The polypeptide of either of claims 13-14, having a molecular weight of approximately 99 kDa.

16. The polypeptide of any of claims 13-15, comprising six transmembrane domains, a P-loop, and a carboxy-terminal conserved cytoplasmic region (the "A-domain").

17. The polypeptide according to claim 13, being a KCNQ5 variant, which variant has an amino acid sequence that has been changed by deletion of an amino acid residue, by insertion of an additional amino acid residue, or by substitution of an amino acid residue at one or more positions.

18. The polypeptide according to claim 17, which variant has an amino acid sequence that has been changed at one or more positions located in the conserved regions, as defined by Table 1.

19. The polypeptide according to claim 18, which variant is G329S (KCNQ1 numbering), or KCNQ5/G278S.

20. A cell genetically manipulated by the incorporation of a heterologous polynucleotide according to any of claims 1-11 or a vector construct according to claim 12.

21. The cell according to claim 20, genetically manipulated by the incorporation of a KCNQ5 channel subunit having the amino acid sequence presented as SEQ ID NO: 2.

22. The cell according to claim 20, genetically manipulated by the incorporation of a KCNQ5 variant, which variant has an amino acid sequence that has been changed by deletion of an amino acid residue, by insertion of an additional amino acid residue, or by substitution of an amino acid residue at one or more positions.

23. The cell according to claim 22, which variant has an amino acid sequence that has been changed at one or more positions located in the conserved regions, as defined by Table 1.

24. The cell according to any of claims 20-23, genetically manipulated to co-express one or more KCNQ channel subunits.

25. The cell according to claim 24, genetically manipulated co-express
KCNQ5 and KCNQ1 channel subunits;
KCNQ5 and KCNQ2 channel subunits;
KCNQ5 and KCNQ3 channel subunits;
KCNQ5 and KCNQ4 channel subunits;
KCNQ5 and KCNQ1 and KCNQ2 channel subunits;
KCNQ5 and KCNQ1 and KCNQ3 channel subunits;
KCNQ5 and KCNQ1 and KCNQ4 channel subunits;
KCNQ5 and KCNQ2 and KCNQ3 channel subunits;
KCNQ5 and KCNQ2 and KCNQ4 channel subunits;
KCNQ5 and KCNQ3 and KCNQ4 channel subunits;
KCNQ5 and KCNQ1 and KCNQ2 and KCNQ3 channel subunits;
KCNQ5 and KCNQ1 and KCNQ2 and KCNQ4 channel subunits;
KCNQ5 and KCNQ1 and KCNQ3 and KCNQ4 channel subunits, or
KCNQ5 and KCNQ2 and KCNQ3 and KCNQ4 channel subunits.

26. The cell according to claim 24, genetically manipulated to co-express KCNQ2 or KCNQ3, and KCNQ5 channel subunits.

27. The cell according to any of claims 20-26, being an eukaryotic cell, in particular a mammalian cell, an oocyte, or a yeast cell.

28. The cell according to any claim 27, being a human embryonic kidney (HEK) cell, a HEK 293 cell, a BHK21 cell, a Chinese hamster ovary (CHO) cell, a *Xenopus laevis* oocyte (XLO) cell, a COS cell, or any other cell line able to express KCNQ potassium channels.

29. A membrane preparation derived from a cell according to any of claims 20-28.

30. A method for obtaining a substantially homogeneous source of a human potassium channel, comprising a KCNQ5 subunit, which method comprises the steps of culturing a cellular host having incorporated expressibly therein a polynucleotide according to any of claims 1-11, or a vector construct according to claim 12, and then recovering the cultured cells.

31. The method of claim 29, comprising the subsequent step of obtaining a membrane preparation from the cultured cells.

32. A method of screening a chemical compound for capability of binding to a potassium channel comprising at least one KCNQ5 channel subunit, which method comprises the steps of

(i) subjecting a KCNQ5 channel subunit containing cell, or a membrane preparation hereof, to the action of a KCNQ5 binding agent to form a complex with the KCNQ5 channel subunit containing cell;

(ii) subjecting the complex of step (i) to the action of the chemical compound to be tested; and

(iii) detecting the displacement of the KCNQ5 binding agent from the complex with the KCNQ5 channel subunit containing cell.

33. The method of claim 32, wherein the KCNQ5 channel subunit containing cell is a cell according to any of claims 20-28, or a membrane preparation according to claim 29.

34. The method of either of claims 32-33, in which the KCNQ5 binding agent is

(i) radioactively labelled 1,3-dihydro-1-phenyl-3,3-bis-(4-pyridylmethyl)-2H-indol-2-one (Linopirdine); or

(ii) radioactively labelled 10,10-bis-(4-pyridinyl-methyl)-9-(10H)-anthracenone (XE991).

35. The method of claim 34, which compounds have been marked with ^{3}H.

36. The method of either of claims 32-33, wherein the displacement of the KCNQ5 binding agent from the complex with the KCNQ5 channel subunit containing cell is detected by measuring the amount of radioactivity by conventional liquid scintillation counting.

37. A method of screening a chemical compound for activity on a potassium channel comprising at least one KCNQ5 channel subunit, which method comprises the steps of

(i) subjecting a KCNQ5 channel subunit containing cell, or a membrane preparation hereof, to the action of the chemical compound; and

(ii) monitoring the membrane potential, the current, the potassium flux, or the secondary calcium influx of the KCNQ5 channel subunit containing cell.

38. The method of claim 37, wherein the KCNQ5 channel subunit containing cell is a cell according to any of claims 20-28, or a membrane preparation according to claim 29.

39. The method of either of claims 37-38, wherein monitoring of the membrane potential of the KCNQ5 channel subunit containing cell is performed by patch clamp techniques.

40. The method of either of claims 37-38, wherein monitoring of the membrane potential of the KCNQ5 channel subunit containing cell is performed using fluorescence methods.

41. A chemical compound identified by the method of claims 32-36 and/or by claims 37-40.

42. Use of the chemical compound according to claim 41 for diagnosis, treatment, prevention or alleviation of diseases related to diseases or adverse conditions of the CNS, including affective disorders, Alzheimer's disease, anxiety, ataxia, CNS damage caused by trauma, stroke or neurodegenerative illness, cognitive deficits, compulsive behaviour, dementia, depression, Huntington's disease, mania, memory impairment, memory disorders, memory dysfunction, motion disorders, motor disorders, neurodegenerative diseases, Parkinson's disease and Parkinson-like motor disorders, phobias, Pick's disease, psychosis, schizophrenia, spinal cord damage, stroke, tremor, seizures, convulsions and epilepsy.

43. The use according to claim 42, wherein the chemical compound is

1,3-dihydro-1-phenyl-3,3-bis-(4-pyridylmethyl)-2H-indol-2-one (Linopirdine); or

10,10-bis-(4-pyridinyl-methyl)-9-(10H)-antracenone (XE991).

44. Use of a polynucleotide sequence according to any of claims 1-11, or a vector construct according to claim 12, for the screening of genetic materials collected from mammalian tissues, in particular human tissues, for individuals having mutations in this gene.

45. A transgenic animal comprising a knock-out mutation of the endogenous *KCNQ5* gene, a mutated *KCNQ5* gene, or genetically manipulated in order to over-express the *KCNQ5* gene or to over-express mutated *KCNQ5* gene.

46. The transgenic animal according to claim 45, being a knock-out animal in which the gene is totally deleted in a homozygous state.

47. The transgenic animal according to claim 45, comprising a mutated *KCNQ5* gene.

48. The transgenic animal according to any of claims 45-47, being a transgenic rodent, in particular a hamster, a guinea pig, a rabbit, or a rat, a transgenic pig, a transgenic cattle, a transgenic sheep, or a transgenic goat.

49. Use of the transgenic animal according to any of claims 45-48 for the *in vivo* screening of therapeutic compounds.

50. The use according to claim 49, for the screening of drugs affecting diseases or conditions associated with malfunction of the CNS, such as affective disorders, Alzheimer's disease, anxiety, ataxia, CNS damage caused by trauma, stroke or neurodegenerative illness, cognitive deficits, compulsive behaviour, dementia, depression, Huntington's disease, mania, memory impairment, memory disorders, memory dysfunction, motion disorders, motor disorders, neurodegenerative diseases, Parkinson's disease and Parkinson-like motor disorders, phobias, Pick's disease, psychosis, schizophrenia, spinal cord damage, stroke, tremor, seizures, convulsions and epilepsy.

51. An antibody capable of binding one or more polypeptides as claimed in any one of claims 13-19.

52. The antibody of claim 51 being a monoclonal antibody.

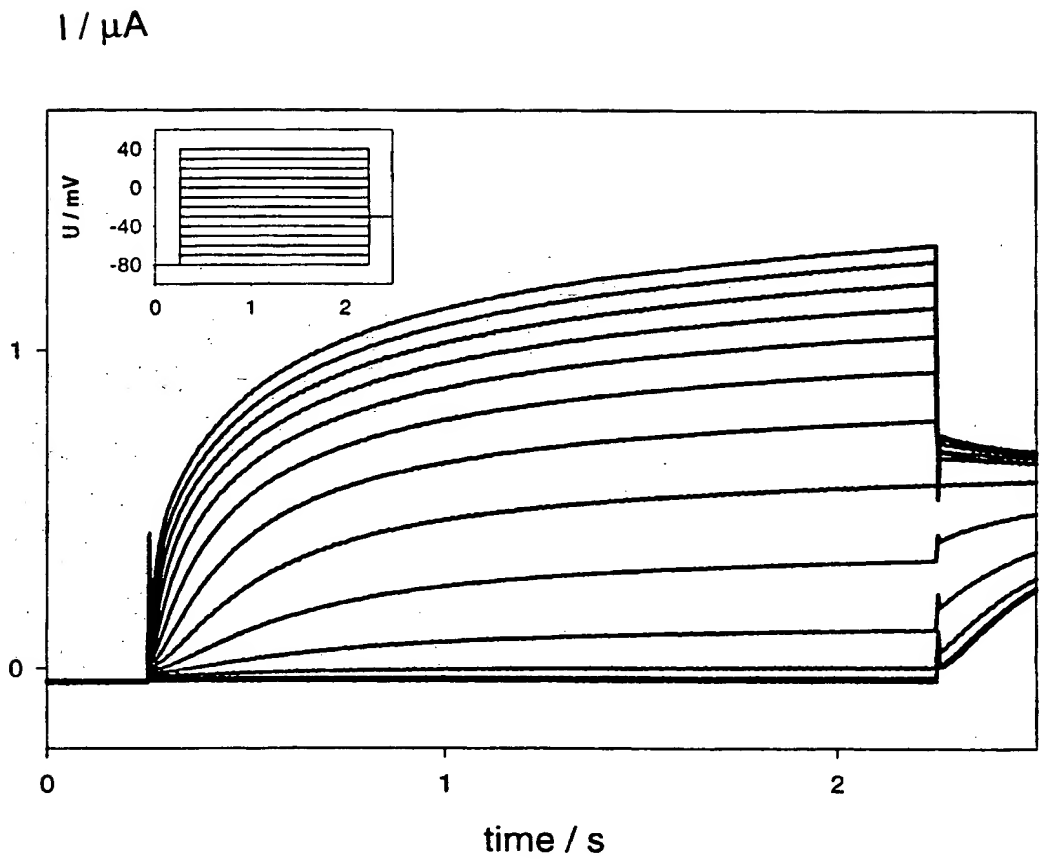


Fig. 1

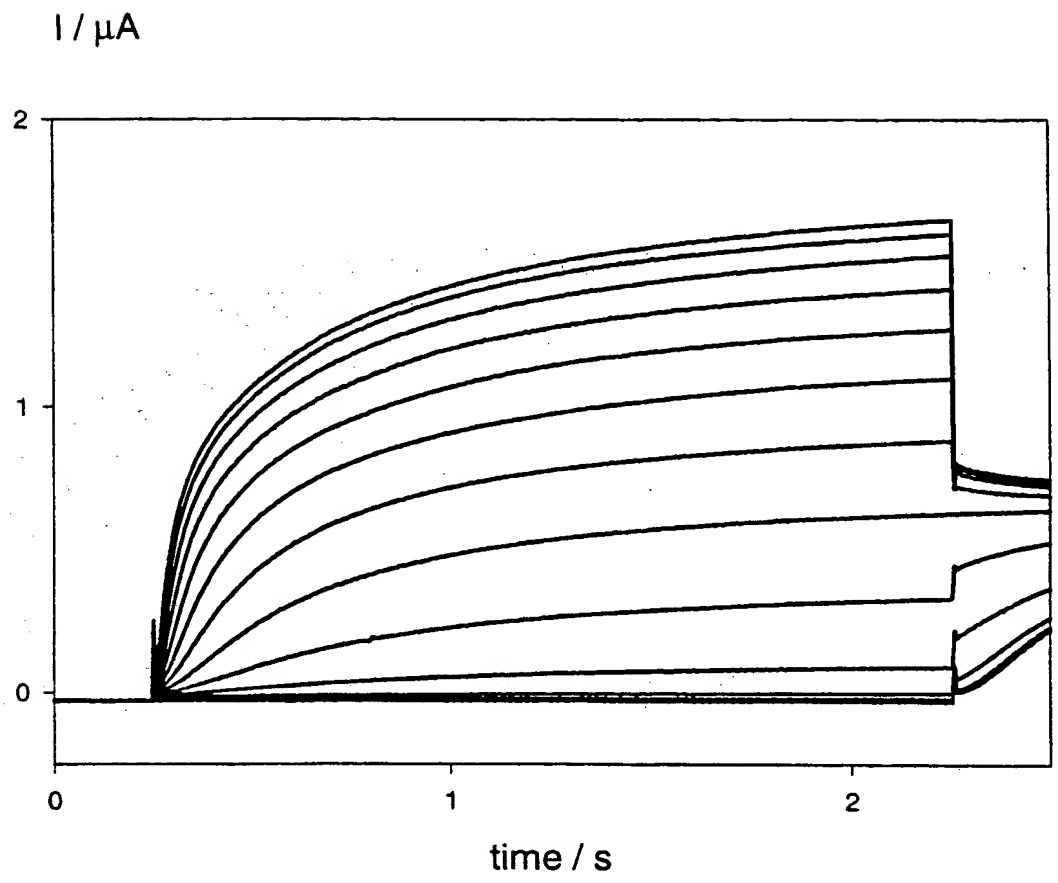


Fig. 2

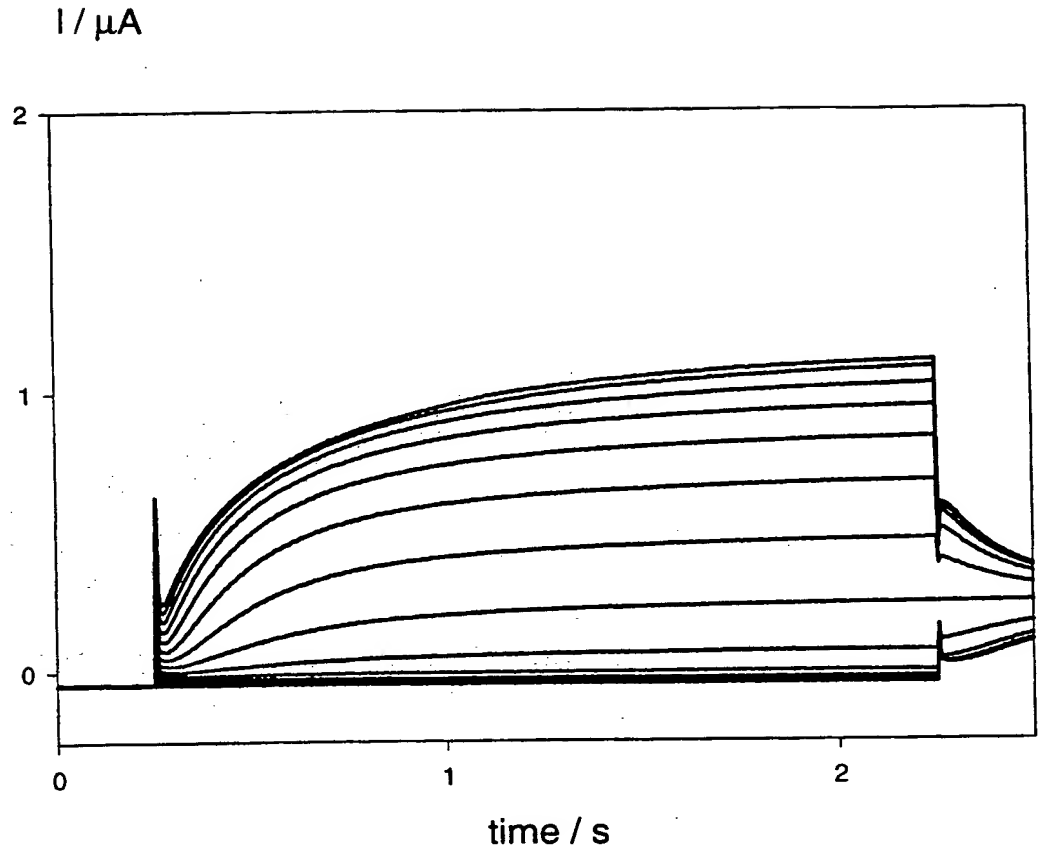
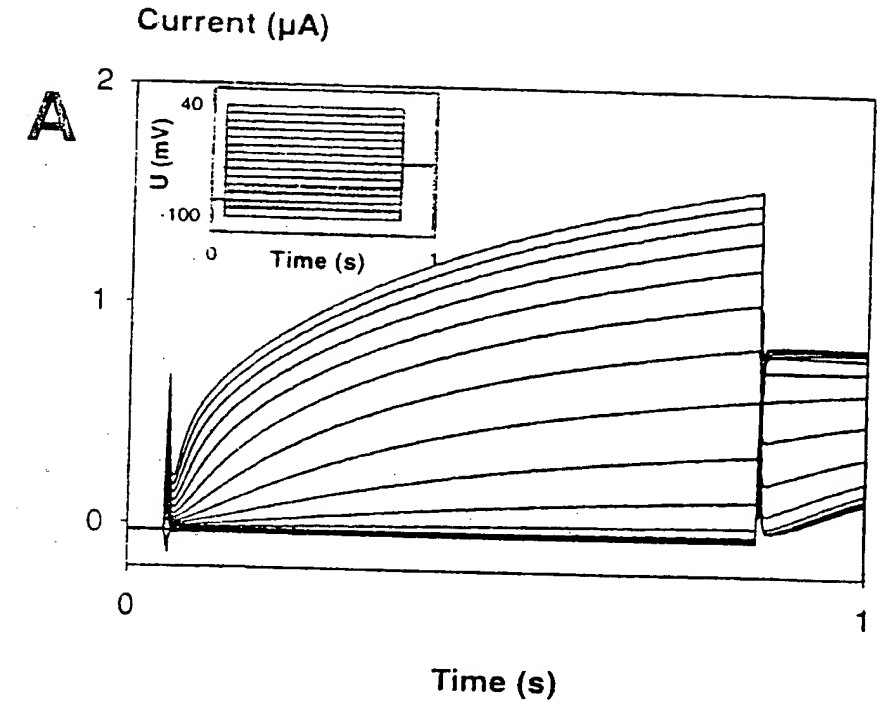


Fig. 3



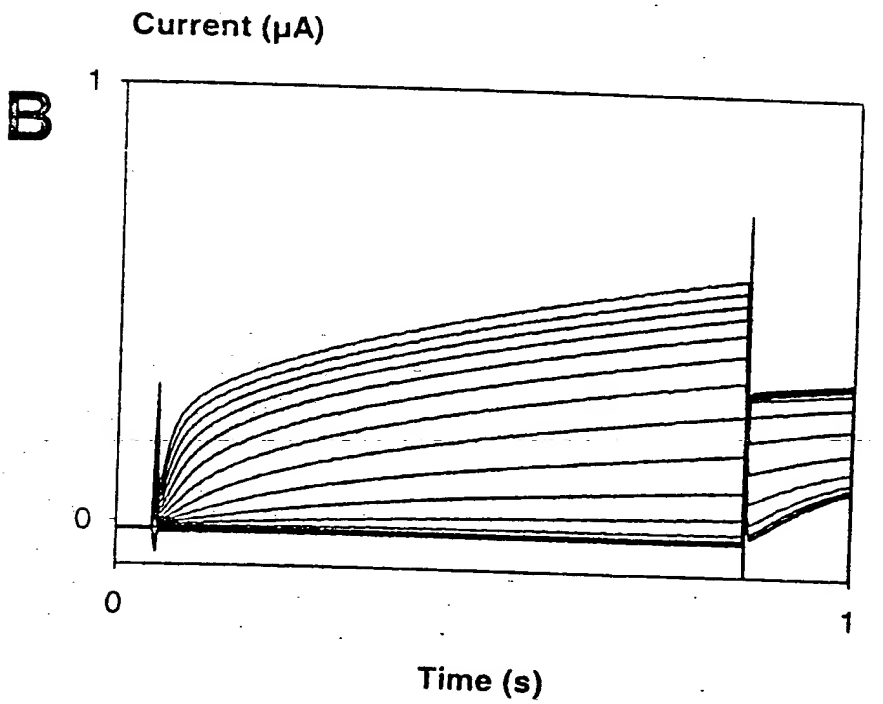
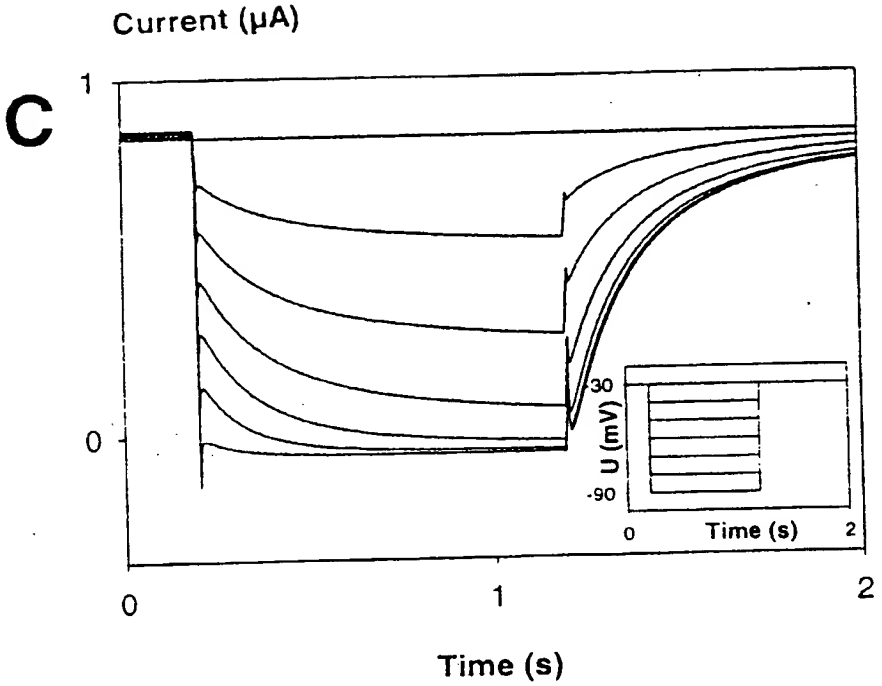


Fig. 4A-B


C
Current (μA)
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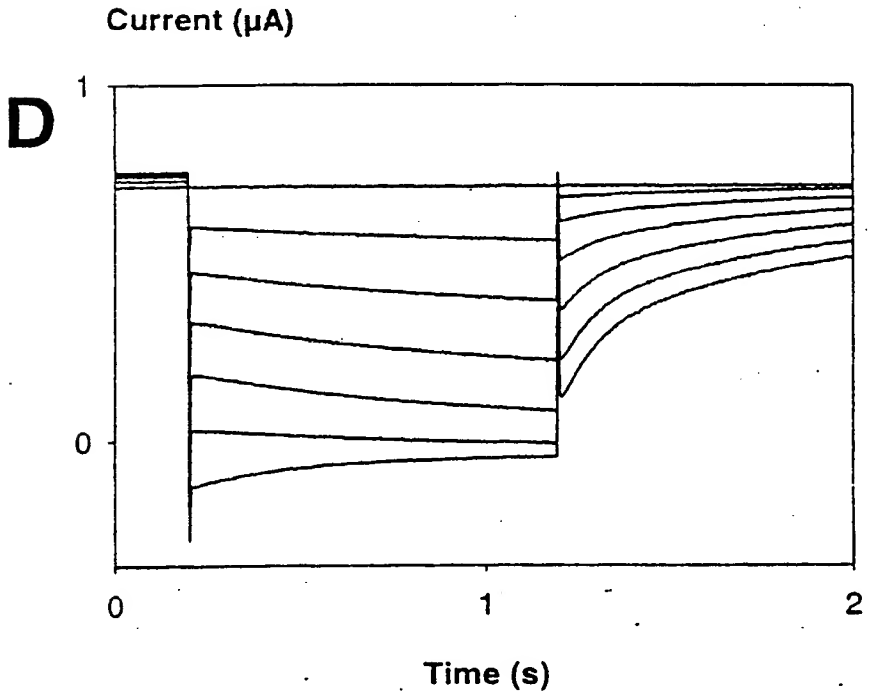
0
0
1
2
Time (s)
30
U (mV)
-90
0
Time (s)
2


D
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0
0
1
2
Time (s)

Fig. 4C-D

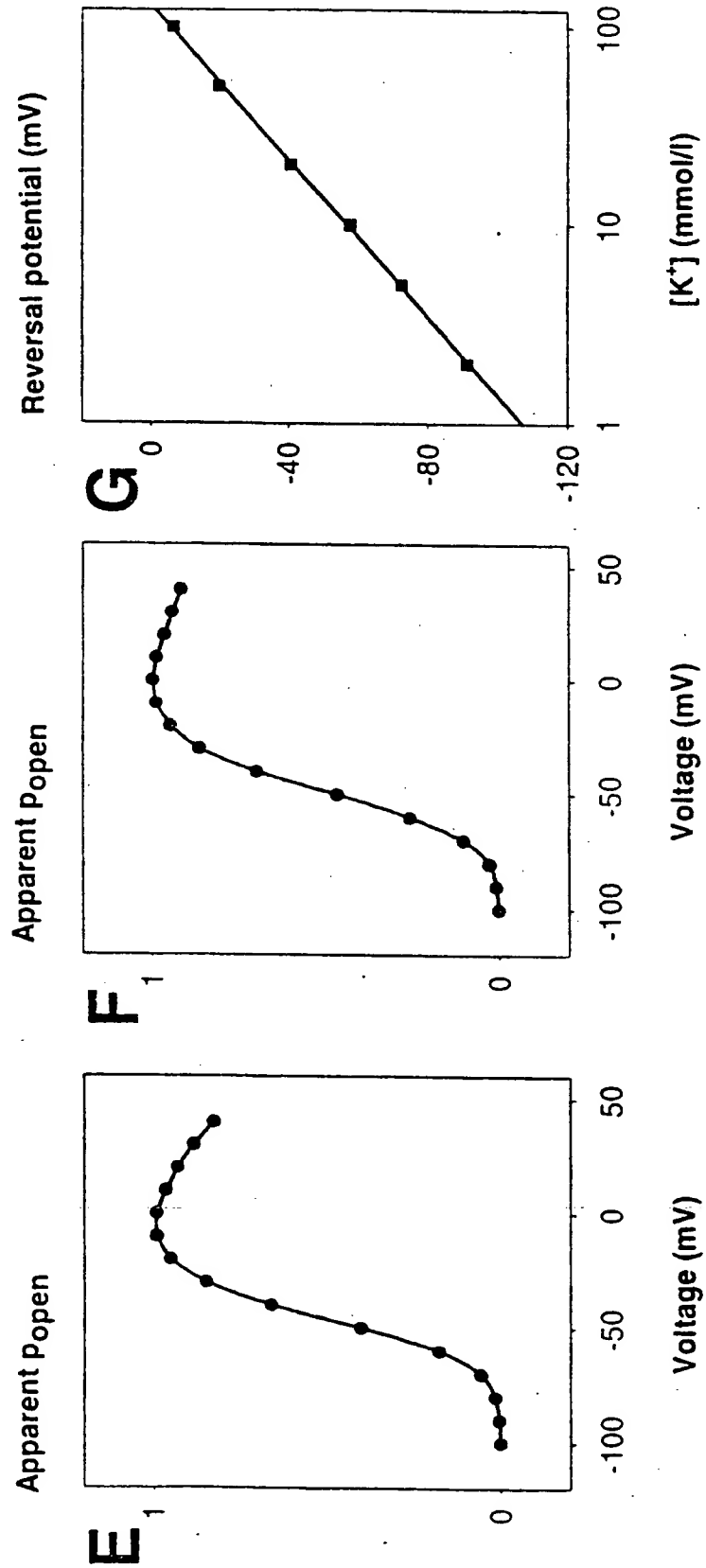


Fig. 4E-G

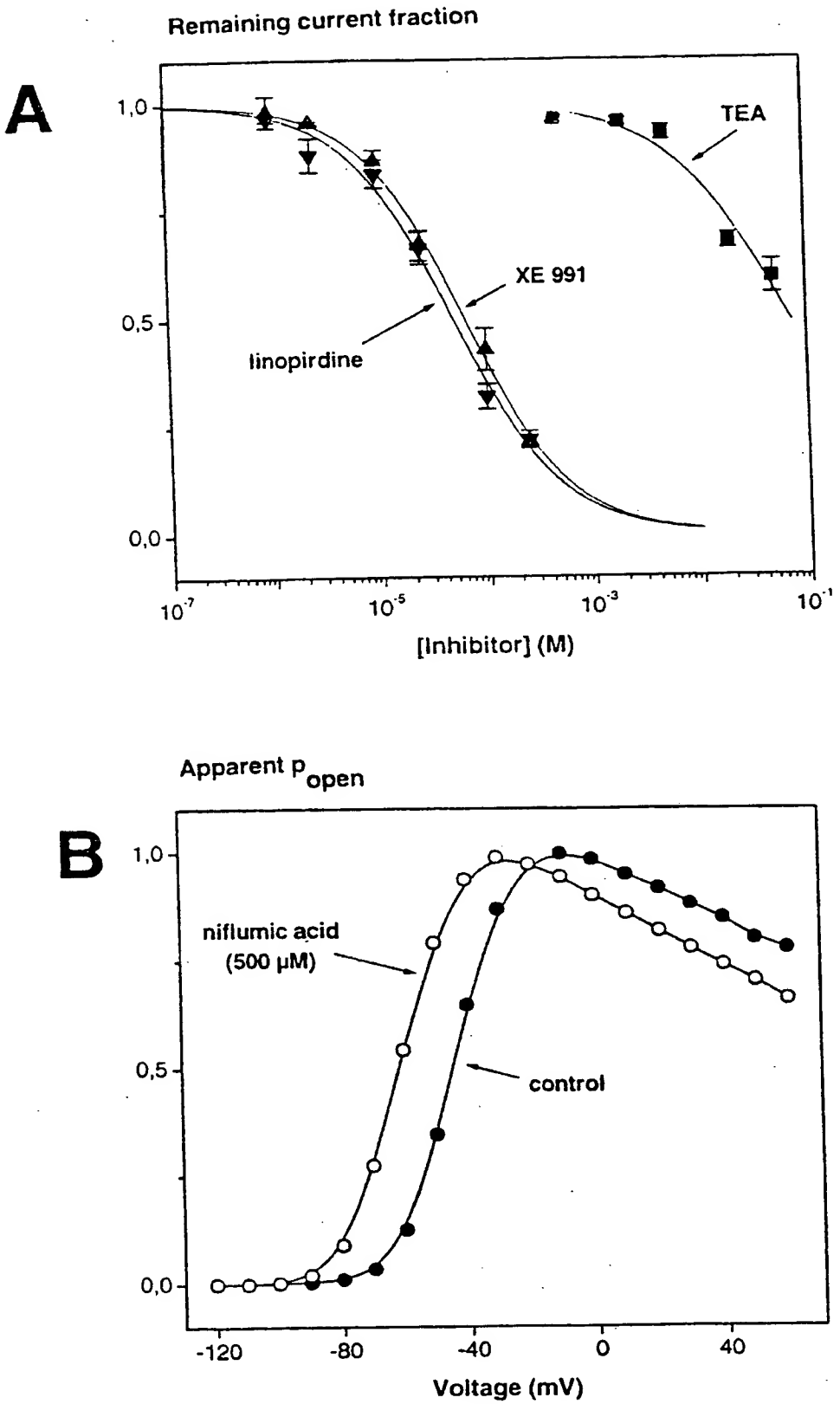

A
Remaining current fraction
1,0
0,5
0,0
TEA
XE 991
linopirdine
10^{-7}
10^{-5}
10^{-3}
10^{-1}
[Inhibitor] (M)
B
Apparent p_{open}
1,0
0,5
0,0
niflumic acid
(500 µM)
control
-120
-80
-40
0
40
Voltage (mV)

Fig. 5A-B

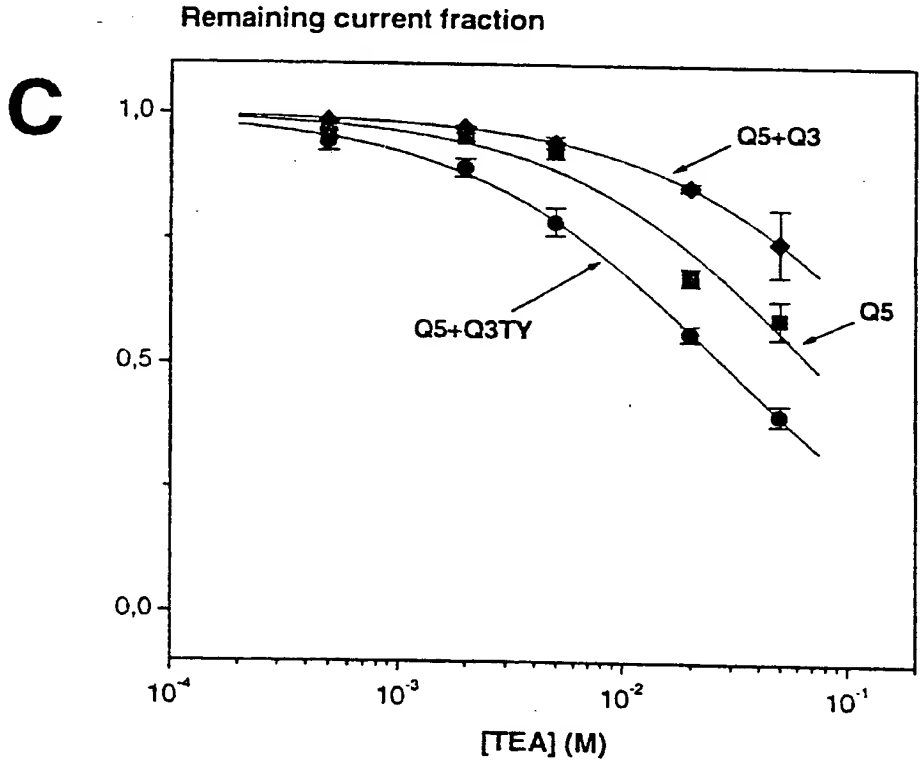
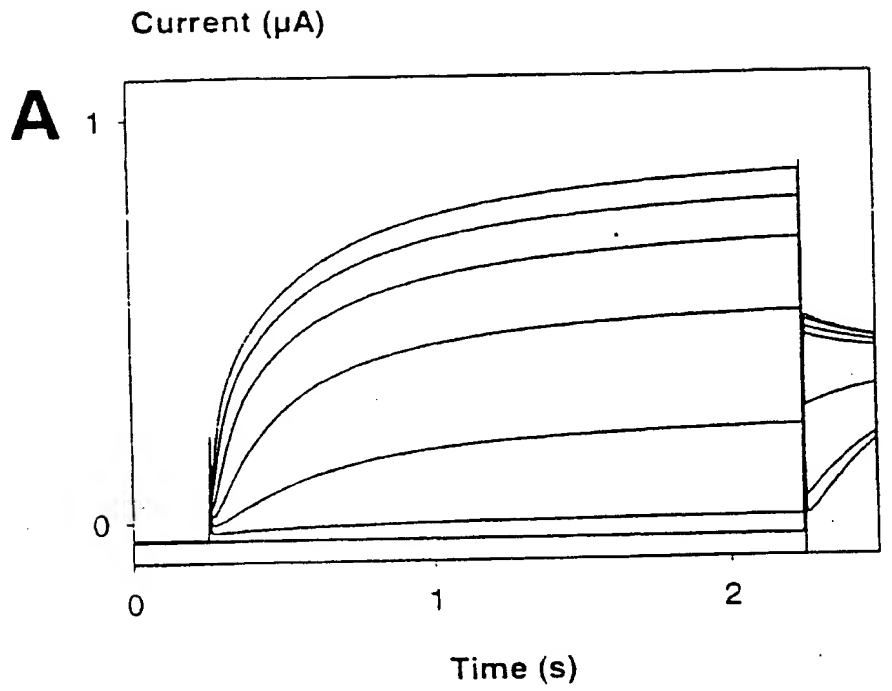


Fig. 5C



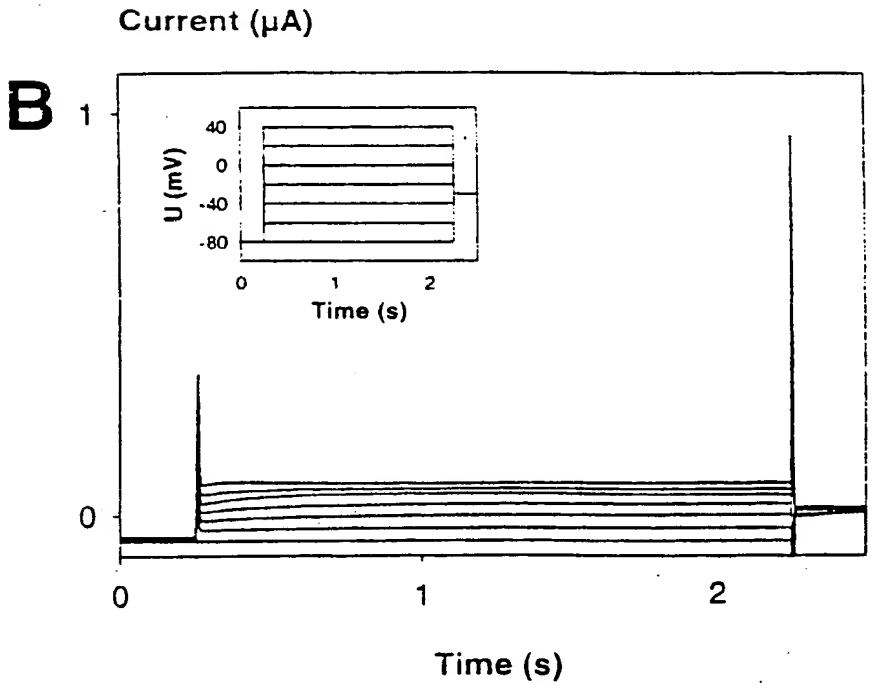


Fig. 6

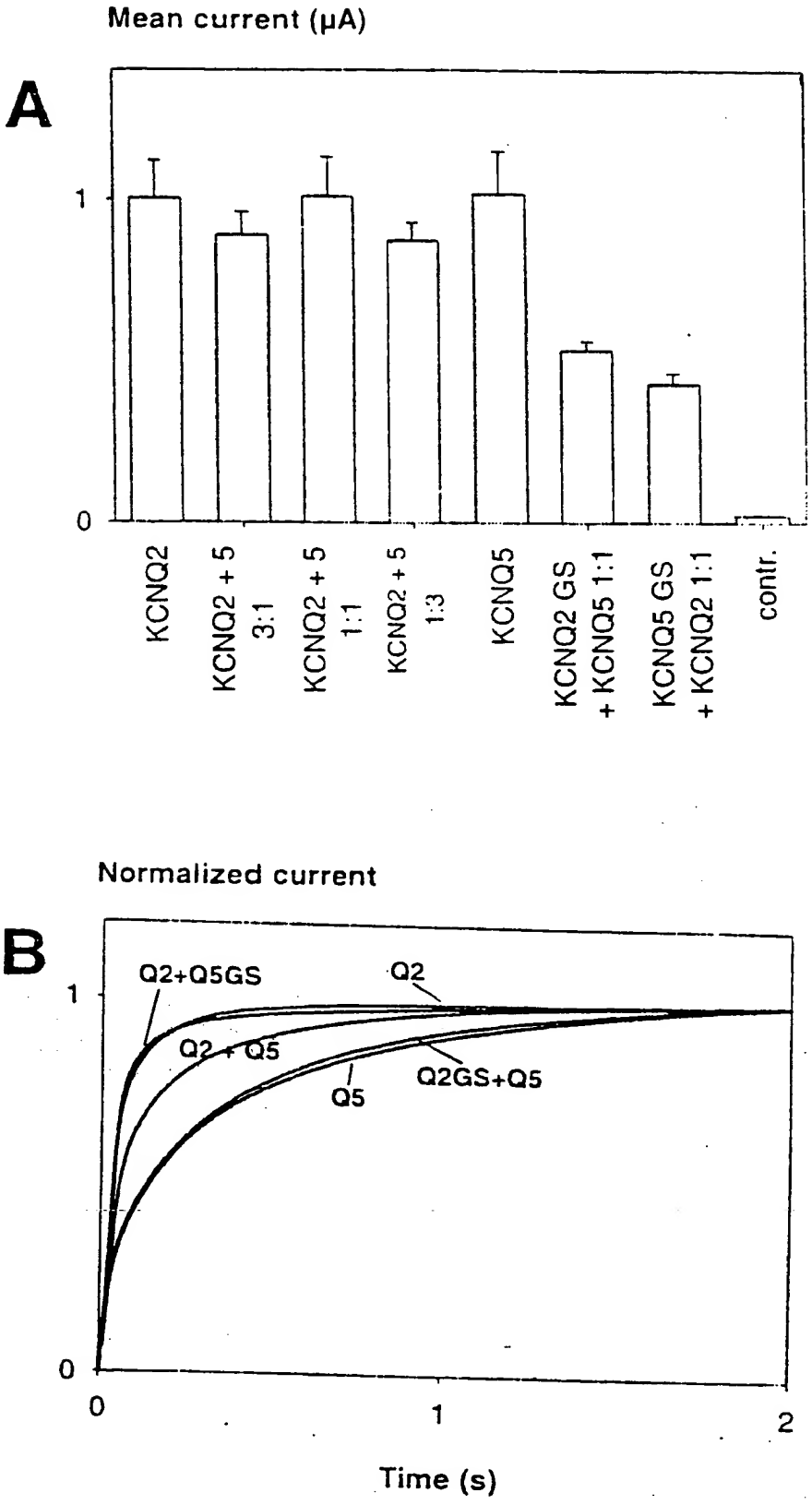


Fig. 7A-B

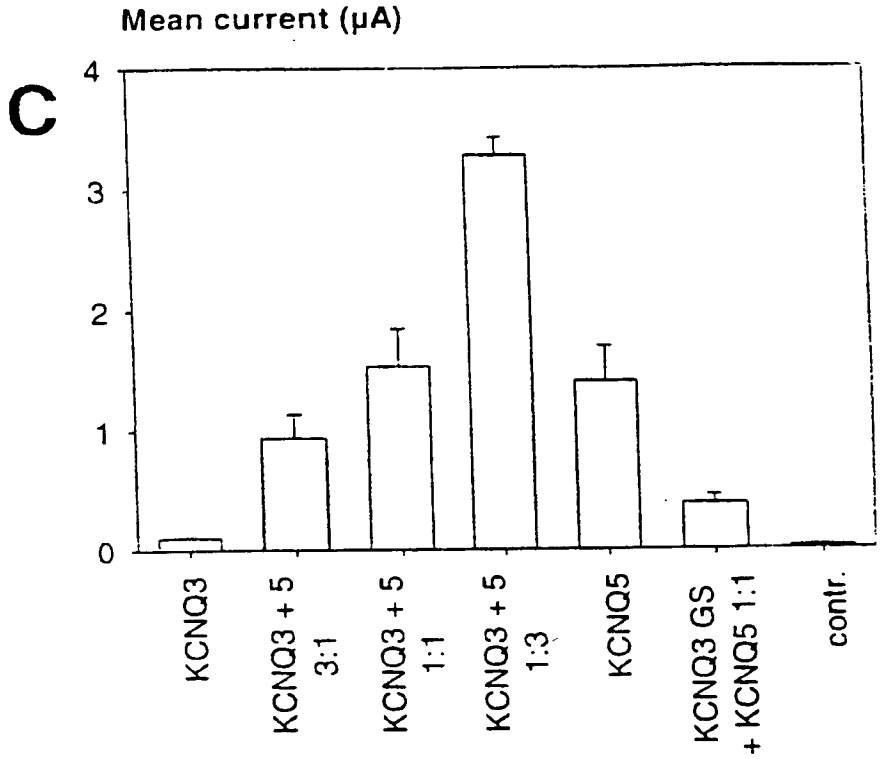


Fig. 7C

SEQUENCE LISTING

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3137 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:

(B) CLONE: KCNQ5

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..2694

(x) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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atg aag gat gtg gag tcg ggc cgg ggc agg gtg ctg ctg aac tcg gca   48
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  1               5                  10                  15

gcc gcc agg ggc gac ggc ctg cta ctg ctg ggc acc cgc gcg gcc acg   96
Ala Ala Arg Gly Asp Gly Leu Leu Leu Leu Gly Thr Arg Ala Ala Thr
            20                  25                  30

ctc ggt ggc ggc ggc ggt ggc ctg agg gag agc cgc cgg ggc aag cag   144
Leu Gly Gly Gly Gly Gly Gly Leu Arg Glu Ser Arg Arg Gly Lys Gln
        35                  40                  45

ggg gcc cgg atg agc ctg ctg ggg aag ccg ctc tct tac acg agt agc   192
Gly Ala Arg Met Ser Leu Leu Gly Lys Pro Leu Ser Tyr Thr Ser Ser
    50                  55                  60

cag agc tgc cgg cgc aac gtc aag tac cgg cgg gtg cag aac tac ctg   240
Gln Ser Cys Arg Arg Asn Val Lys Tyr Arg Arg Val Gln Asn Tyr Leu
65                  70                  75                  80

tac aac gtg ctg gag aga ccc cgc ggc tgg gcg ttc atc tac cac gct   288
Tyr Asn Val Leu Glu Arg Pro Arg Gly Trp Ala Phe Ile Tyr His Ala
                85                  90                  95

ttc gtt ttt ctc ctt gtc ttt ggt tgc ttg att ttg tca gtg ttt tct   336
Phe Val Phe Leu Leu Val Phe Gly Cys Leu Ile Leu Ser Val Phe Ser
            100                 105                 110

acc atc cct gag cac aca aaa ttg gcc tca agt tgc ctc ttg atc ctg   384
Thr Ile Pro Glu His Thr Lys Leu Ala Ser Ser Cys Leu Leu Ile Leu
        115                 120                 125

gag ttc gtg atg att gtc gtc ttt ggt ttg gag ttc atc att cga atc   432
Glu Phe Val Met Ile Val Val Phe Gly Leu Glu Phe Ile Ile Arg Ile
    130                 135                 140
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Trp Ser Ala Gly Cys Cys Cys Arg Tyr Arg Gly Trp Gln Gly Arg Leu
145                 150                 155                 160

agg ttt gct cga aag ccc ttc tgt gtt ata gat acc att gtt ctt atc   528
Arg Phe Ala Arg Lys Pro Phe Cys Val Ile Asp Thr Ile Val Leu Ile
                165                 170                 175

gct tca ata gca gtt gtt tct gca aaa act cag ggt aat att ttt gcc   576
Ala Ser Ile Ala Val Val Ser Ala Lys Thr Gln Gly Asn Ile Phe Ala
            180                 185                 190

acg tct gca ctc aga agt ctc cgt ttc cta cag atc ctc cgc atg gtg   624
Thr Ser Ala Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg Met Val
        195                 200                 205

cgc atg gac cga agg gga ggc act tgg aaa tta ctg ggt tca gtg gtt   672
Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser Val Val
    210                 215                 220

tat gct cac agc aag gaa tta atc aca gct tgg tac ata gga ttt ttg   720
Tyr Ala His Ser Lys Glu Leu Ile Thr Ala Trp Tyr Ile Gly Phe Leu
225                 230                 235                 240

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Val Leu Ile Phe Ser Ser Phe Leu Val Tyr Leu Val Glu Lys Asp Ala
                245                 250                 255

aat aaa gag ttt tct aca tat gca gat gct ctc tgg tgg ggc aca att   816
Asn Lys Glu Phe Ser Thr Tyr Ala Asp Ala Leu Trp Trp Gly Thr Ile
            260                 265                 270

aca ttg aca act att ggc tat gga gac aaa act ccc cta act tgg ctg   864
Thr Leu Thr Thr Ile Gly Tyr Gly Asp Lys Thr Pro Leu Thr Trp Leu
        275                 280                 285

gga aga ttg ctt tct gca ggc ttt gca ctc ctt ggc att tct ttc ttt   912
Gly Arg Leu Leu Ser Ala Gly Phe Ala Leu Leu Gly Ile Ser Phe Phe
    290                 295                 300

gca ctt cct gcc ggc att ctt ggc tca ggt ttt gca tta aaa gta caa   960
Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala Leu Lys Val Gln
305                 310                 315                 320

gaa caa cac cgc cag aaa cac ttt gag aaa aga agg aac cca gct gcc  1008
Glu Gln His Arg Gln Lys His Phe Glu Lys Arg Arg Asn Pro Ala Ala
                325                 330                 335

aac ctc att cag tgt gtt tgg cgt agt tac gca gct gat gag aaa tct  1056
Asn Leu Ile Gln Cys Val Trp Arg Ser Tyr Ala Ala Asp Glu Lys Ser
            340                 345                 350

gtt tcc att gca acc tgg aag cca cac ttg aag gcc ttg cac acc tgc  1104
Val Ser Ile Ala Thr Trp Lys Pro His Leu Lys Ala Leu His Thr Cys
        355                 360                 365

agc cct acc aag aaa gaa caa ggg gaa gca tca agc agt cag aag cta  1152
Ser Pro Thr Lys Lys Glu Gln Gly Glu Ala Ser Ser Ser Gln Lys Leu
    370                 375                 380

agt ttt aag gag cga gtg cgc atg gct agc ccc agg ggc cag agt att  1200
Ser Phe Lys Glu Arg Val Arg Met Ala Ser Pro Arg Gly Gln Ser Ile
385                 390                 395                 400
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Lys Ser Arg Gln Ala Ser Val Gly Asp Arg Arg Ser Pro Ser Thr Asp
                405                 410                 415

atc aca gcc gag ggc agt ccc acc aaa gtg cag aag agc tgg agc ttc   1296
Ile Thr Ala Glu Gly Ser Pro Thr Lys Val Gln Lys Ser Trp Ser Phe
            420                 425                 430

aac gac cga acc cgc ttc cgg ccc tcg ctg cgc ctc aaa agt tct cag   1344
Asn Asp Arg Thr Arg Phe Arg Pro Ser Leu Arg Leu Lys Ser Ser Gln
        435                 440                 445

cca aaa cca gtg ata gat gct gac aca gcc ctt ggc act gat gat gta   1392
Pro Lys Pro Val Ile Asp Ala Asp Thr Ala Leu Gly Thr Asp Asp Val
    450                 455                 460

tat gat gaa aaa gga tgc cag tgt gat gta tca gtg gaa gac ctc acc   1440
Tyr Asp Glu Lys Gly Cys Gln Cys Asp Val Ser Val Glu Asp Leu Thr
465                 470                 475                 480

cca cca ctt aaa act gtc att cga gct atc aga att atg aaa ttt cat   1488
Pro Pro Leu Lys Thr Val Ile Arg Ala Ile Arg Ile Met Lys Phe His
                485                 490                 495

gtt gca aaa cgg aag ttt aag gaa aca tta cgt cca tat gat gta aaa   1536
Val Ala Lys Arg Lys Phe Lys Glu Thr Leu Arg Pro Tyr Asp Val Lys
            500                 505                 510

gat gtc att gaa caa tat tct gct ggt cat ctg gac atg ttg tgt aga   1584
Asp Val Ile Glu Gln Tyr Ser Ala Gly His Leu Asp Met Leu Cys Arg
        515                 520                 525

att aaa agc ctt caa aca cgt gtt gat caa att ctt gga aaa ggg caa   1632
Ile Lys Ser Leu Gln Thr Arg Val Asp Gln Ile Leu Gly Lys Gly Gln
    530                 535                 540

atc aca tca gat aag aag agc cga gag aaa ata aca gca gaa cat gag   1680
Ile Thr Ser Asp Lys Lys Ser Arg Glu Lys Ile Thr Ala Glu His Glu
545                 550                 555                 560

acc aca gac gat ctc agt atg ctc ggt cgg gtg gtc aag gtt gaa aaa   1728
Thr Thr Asp Asp Leu Ser Met Leu Gly Arg Val Val Lys Val Glu Lys
                565                 570                 575

cag gta cag tcc ata gaa tcc aag ctg gac tgc cta cta gac atc tat   1776
Gln Val Gln Ser Ile Glu Ser Lys Leu Asp Cys Leu Leu Asp Ile Tyr
            580                 585                 590

caa cag gtc ctt cgg aaa ggc tct gcc tca gcc ctc gct ttg gct tca   1824
Gln Gln Val Leu Arg Lys Gly Ser Ala Ser Ala Leu Ala Leu Ala Ser
        595                 600                 605

ttc cag atc cca cct ttt gaa tgt gaa cag aca tct gac tat caa agc   1872
Phe Gln Ile Pro Pro Phe Glu Cys Glu Gln Thr Ser Asp Tyr Gln Ser
    610                 615                 620

cct gtg gat agc aaa gat ctt tcg ggt tcc gca caa aac agt ggc tgc   1920
Pro Val Asp Ser Lys Asp Leu Ser Gly Ser Ala Gln Asn Ser Gly Cys
625                 630                 635                 640

tta tcc aga tca act agt gcc aac atc tcg aga ggc ctg cag ttc att   1968
Leu Ser Arg Ser Thr Ser Ala Asn Ile Ser Arg Gly Leu Gln Phe Ile
                645                 650                 655
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ctg acg cca aat gag ttc agt gcc cag act ttc tac gcg ctt agc cct   2016
Leu Thr Pro Asn Glu Phe Ser Ala Gln Thr Phe Tyr Ala Leu Ser Pro
            660                 665                 670

act atg cac agt caa gca aca cag gtg cca att agt caa agc gat ggc   2064
Thr Met His Ser Gln Ala Thr Gln Val Pro Ile Ser Gln Ser Asp Gly
        675                 680                 685

tca gca gtg gca gcc acc aac acc att gca aac caa ata aat acg gca   2112
Ser Ala Val Ala Ala Thr Asn Thr Ile Ala Asn Gln Ile Asn Thr Ala
    690                 695                 700

ccc aag cca gca gcc cca aca act tta cag atc cca cct cct ctc cca   2160
Pro Lys Pro Ala Ala Pro Thr Thr Leu Gln Ile Pro Pro Pro Leu Pro
705                 710                 715                 720

gcc atc aag cat ctg ccc agg cca gaa act ctg cac cct aac cct gca   2208
Ala Ile Lys His Leu Pro Arg Pro Glu Thr Leu His Pro Asn Pro Ala
                725                 730                 735

ggc tta cag gaa agc att tct gac gtc acc acc tgc ctt gtt gcc tcc   2256
Gly Leu Gln Glu Ser Ile Ser Asp Val Thr Thr Cys Leu Val Ala Ser
            740                 745                 750

aag gaa aat gtt cag gtt gca cag tca aat ctc acc aag gac cgt tct   2304
Lys Glu Asn Val Gln Val Ala Gln Ser Asn Leu Thr Lys Asp Arg Ser
        755                 760                 765

atg agg aaa agc ttt gac atg gga gga gaa act ctg ttg tct gtc tgt   2352
Met Arg Lys Ser Phe Asp Met Gly Gly Glu Thr Leu Leu Ser Val Cys
    770                 775                 780

ccc atg gtg ccg aag gac ttg ggc aaa tct ttg tct gtg caa aac ctg   2400
Pro Met Val Pro Lys Asp Leu Gly Lys Ser Leu Ser Val Gln Asn Leu
785                 790                 795                 800

atc agg tcg acc gag gaa ctg aat ata caa ctt tca ggg agt gag tca   2448
Ile Arg Ser Thr Glu Glu Leu Asn Ile Gln Leu Ser Gly Ser Glu Ser
                805                 810                 815

agt ggc tcc aga ggc agc caa gat ttt tac ccc aaa tgg agg gaa tcc   2496
Ser Gly Ser Arg Gly Ser Gln Asp Phe Tyr Pro Lys Trp Arg Glu Ser
            820                 825                 830

aaa ttg ttt ata act gat gaa gag gtg ggt ccc gaa gag aca gag aca   2544
Lys Leu Phe Ile Thr Asp Glu Glu Val Gly Pro Glu Glu Thr Glu Thr
        835                 840                 845

gac act ttt gat gcc gca ccg cag cct gcc agg gaa gct gcc ttt gca   2592
Asp Thr Phe Asp Ala Ala Pro Gln Pro Ala Arg Glu Ala Ala Phe Ala
    850                 855                 860

tca gac tct cta agg act gga agg tca cga tca tct cag agc att tgt   2640
Ser Asp Ser Leu Arg Thr Gly Arg Ser Arg Ser Ser Gln Ser Ile Cys
865                 870                 875                 880

aag gca gga gaa agt aca gat gcc ctc agc ttg cct cat gtc aaa ctg   2688
Lys Ala Gly Glu Ser Thr Asp Ala Leu Ser Leu Pro His Val Lys Leu
                885                 890                 895

aaa taagttcttc attttctttc caggcatagc agttctttag ccatacatat        2741
Lys

cattgcatga actatttcga aagcccttct aaaaagttga aattgcaaga atcgggaaga 2801

acatgaaagg cagtttataa gcccgttacc ttttaattgc atgaaaatgc atgtttaggg 2861
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atggctaaaa ttccaaggtg catcgacatt aacccactca tttagtaatg taccttgagt 2921

taaaaagcct gagaaaccaa acacagctaa tgctatgggg tgtatgaata tgtcaagttt 2981

aggtcattta gaagatttga cactgtattt tgaaattatg ggagtaaaca ccttcaaatt 3041

tcaggcattt ctgctttgtg actaaataca aactacattt tcaagattag gccataatgt 3101

atatttaaac acaatggcta tcaacagctg ctaata 3137

2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 897 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Lys Asp Val Glu Ser Gly Arg Gly Arg Val Leu Leu Asn Ser Ala
1 5 10 15

Ala Ala Arg Gly Asp Gly Leu Leu Leu Leu Gly Thr Arg Ala Ala Thr
20 25 30

Leu Gly Gly Gly Gly Gly Gly Leu Arg Glu Ser Arg Arg Gly Lys Gln
35 40 45

Gly Ala Arg Met Ser Leu Leu Gly Lys Pro Leu Ser Tyr Thr Ser Ser
50 55 60

Gln Ser Cys Arg Arg Asn Val Lys Tyr Arg Arg Val Gln Asn Tyr Leu
65 70 75 80

Tyr Asn Val Leu Glu Arg Pro Arg Gly Trp Ala Phe Ile Tyr His Ala
85 90 95

Phe Val Phe Leu Leu Val Phe Gly Cys Leu Ile Leu Ser Val Phe Ser
100 105 110

Thr Ile Pro Glu His Thr Lys Leu Ala Ser Ser Cys Leu Leu Ile Leu
115 120 125

Glu Phe Val Met Ile Val Val Phe Gly Leu Glu Phe Ile Ile Arg Ile
130 135 140

Trp Ser Ala Gly Cys Cys Cys Arg Tyr Arg Gly Trp Gln Gly Arg Leu
145 150 155 160

Arg Phe Ala Arg Lys Pro Phe Cys Val Ile Asp Thr Ile Val Leu Ile
165 170 175

Ala Ser Ile Ala Val Val Ser Ala Lys Thr Gln Gly Asn Ile Phe Ala
180 185 190

Thr Ser Ala Leu Arg Ser Leu Arg Phe Leu Gln Ile Leu Arg Met Val
195 200 205

Arg Met Asp Arg Arg Gly Gly Thr Trp Lys Leu Leu Gly Ser Val Val
210 215 220

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Tyr Ala His Ser Lys Glu Leu Ile Thr Ala Trp Tyr Ile Gly Phe Leu
225                 230                 235                 240

Val Leu Ile Phe Ser Ser Phe Leu Val Tyr Leu Val Glu Lys Asp Ala
                245                 250                 255

Asn Lys Glu Phe Ser Thr Tyr Ala Asp Ala Leu Trp Trp Gly Thr Ile
            260                 265                 270

Thr Leu Thr Thr Ile Gly Tyr Gly Asp Lys Thr Pro Leu Thr Trp Leu
        275                 280                 285

Gly Arg Leu Leu Ser Ala Gly Phe Ala Leu Leu Gly Ile Ser Phe Phe
    290                 295                 300

Ala Leu Pro Ala Gly Ile Leu Gly Ser Gly Phe Ala Leu Lys Val Gln
305                 310                 315                 320

Glu Gln His Arg Gln Lys His Phe Glu Lys Arg Arg Asn Pro Ala Ala
                325                 330                 335

Asn Leu Ile Gln Cys Val Trp Arg Ser Tyr Ala Ala Asp Glu Lys Ser
            340                 345                 350

Val Ser Ile Ala Thr Trp Lys Pro His Leu Lys Ala Leu His Thr Cys
        355                 360                 365

Ser Pro Thr Lys Lys Glu Gln Gly Glu Ala Ser Ser Ser Gln Lys Leu
    370                 375                 380

Ser Phe Lys Glu Arg Val Arg Met Ala Ser Pro Arg Gly Gln Ser Ile
385                 390                 395                 400

Lys Ser Arg Gln Ala Ser Val Gly Asp Arg Arg Ser Pro Ser Thr Asp
                405                 410                 415

Ile Thr Ala Glu Gly Ser Pro Thr Lys Val Gln Lys Ser Trp Ser Phe
            420                 425                 430

Asn Asp Arg Thr Arg Phe Arg Pro Ser Leu Arg Leu Lys Ser Ser Gln
        435                 440                 445

Pro Lys Pro Val Ile Asp Ala Asp Thr Ala Leu Gly Thr Asp Asp Val
    450                 455                 460

Tyr Asp Glu Lys Gly Cys Gln Cys Asp Val Ser Val Glu Asp Leu Thr
465                 470                 475                 480

Pro Pro Leu Lys Thr Val Ile Arg Ala Ile Arg Ile Met Lys Phe His
                485                 490                 495

Val Ala Lys Arg Lys Phe Lys Glu Thr Leu Arg Pro Tyr Asp Val Lys
            500                 505                 510

Asp Val Ile Glu Gln Tyr Ser Ala Gly His Leu Asp Met Leu Cys Arg
        515                 520                 525

Ile Lys Ser Leu Gln Thr Arg Val Asp Gln Ile Leu Gly Lys Gly Gln
    530                 535                 540

Ile Thr Ser Asp Lys Lys Ser Arg Glu Lys Ile Thr Ala Glu His Glu
545                 550                 555                 560

Thr Thr Asp Asp Leu Ser Met Leu Gly Arg Val Val Lys Val Glu Lys
                565                 570                 575
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Gln Val Gln Ser Ile Glu Ser Lys Leu Asp Cys Leu Leu Asp Ile Tyr
            580                 585                 590

Gln Gln Val Leu Arg Lys Gly Ser Ala Ser Ala Leu Ala Leu Ala Ser
        595                 600                 605

Phe Gln Ile Pro Pro Phe Glu Cys Glu Gln Thr Ser Asp Tyr Gln Ser
    610                 615                 620

Pro Val Asp Ser Lys Asp Leu Ser Gly Ser Ala Gln Asn Ser Gly Cys
625                 630                 635                 640

Leu Ser Arg Ser Thr Ser Ala Asn Ile Ser Arg Gly Leu Gln Phe Ile
                645                 650                 655

Leu Thr Pro Asn Glu Phe Ser Ala Gln Thr Phe Tyr Ala Leu Ser Pro
            660                 665                 670

Thr Met His Ser Gln Ala Thr Gln Val Pro Ile Ser Gln Ser Asp Gly
        675                 680                 685

Ser Ala Val Ala Ala Thr Asn Thr Ile Ala Asn Gln Ile Asn Thr Ala
    690                 695                 700

Pro Lys Pro Ala Ala Pro Thr Thr Leu Gln Ile Pro Pro Pro Leu Pro
705                 710                 715                 720

Ala Ile Lys His Leu Pro Arg Pro Glu Thr Leu His Pro Asn Pro Ala
                725                 730                 735

Gly Leu Gln Glu Ser Ile Ser Asp Val Thr Thr Cys Leu Val Ala Ser
            740                 745                 750

Lys Glu Asn Val Gln Val Ala Gln Ser Asn Leu Thr Lys Asp Arg Ser
        755                 760                 765

Met Arg Lys Ser Phe Asp Met Gly Gly Glu Thr Leu Leu Ser Val Cys
    770                 775                 780

Pro Met Val Pro Lys Asp Leu Gly Lys Ser Leu Ser Val Gln Asn Leu
785                 790                 795                 800

Ile Arg Ser Thr Glu Glu Leu Asn Ile Gln Leu Ser Gly Ser Glu Ser
                805                 810                 815

Ser Gly Ser Arg Gly Ser Gln Asp Phe Tyr Pro Lys Trp Arg Glu Ser
            820                 825                 830

Lys Leu Phe Ile Thr Asp Glu Glu Val Gly Pro Glu Glu Thr Glu Thr
        835                 840                 845

Asp Thr Phe Asp Ala Ala Pro Gln Pro Ala Arg Glu Ala Ala Phe Ala
    850                 855                 860

Ser Asp Ser Leu Arg Thr Gly Arg Ser Arg Ser Ser Gln Ser Ile Cys
865                 870                 875                 880

Lys Ala Gly Glu Ser Thr Asp Ala Leu Ser Leu Pro His Val Lys Leu
                885                 890                 895

Lys
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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
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PCT

(10) International Publication Number
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(71) Applicant: NEUROSEARCH A/S [DK/DK]; 93 Pederstrupvej, DK-2750 Ballerup (DK).

(72) Inventor: JENTSCH, Thomas, J.; Zentrum für Molekulare Neurobiologie (ZMNH), Universität Hamburg, Falkenried 94, D-20251 Hamburg (DE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

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10 May 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/77035 A3

(54) Title: NOVEL POTASSIUM CHANNELS AND GENES ENCODING THESE POTASSIUM CHANNELS

(57) Abstract: This invention relates to novel potassium channels and genes encoding these channels. More specifically the invention provides isolated polynucleotides encoding the KCNQ5 potassium channel subunit, cells transformed with these polynucleotides, transgenic animals comprising genetic mutations, and the use of the transformed cells and the transgenic animals for the *in vitro* and *in vivo* screening of chemical compounds affecting KCNQ5 subunit containing potassium channels.

INTERNATIONAL SEARCH REPORT

Inter ›nal Application No
PCi/DK 00/00289

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/705 C12N15/12 C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EMBL, EPO-Internal, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	DATABASE EMBL/GENBANK [Online] 14 May 2000 (2000-05-14) SCHROEDER B C ET AL: "KCNQ5, a novel potassium channel broadly expressed in brain, mediates M-type currents" Database accession no. AF202977 XP002901374 abstract Unpublished --- -/--	1-40, 44-52

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 December 2000	20.02.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	P. Andersson

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KUBISCH CH ET AL: "KCNQ4, a novel potassium channel expressed in sensory outer hair cells, is mutated in dominant deafness" CELL, vol. 96, no. 5, 5 February 1999 (1999-02-05), pages 437-446, XP002901375 page 442, column 1, line 1 - line 5; figure 4 the whole document	1-40, 43-52
X	WO 99 07832 A (SQUIBB BRISTOL MYERS CO) 18 February 1999 (1999-02-18) the whole document	1-40, 44-52
X	WO 99 21875 A (UNIV UTAH RES FOUND) 6 May 1999 (1999-05-06) the whole document	1-40, 44-52
X	DATABASE MEDLINE [Online] US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; TAM S W ET AL: "Linopirdine. A depolarization-activated releaser of transmitters for treatment of dementia" Database accession no. 95343820 XP002900987 abstract & ADV EXP MED BIOL, no. 363, 1995, pages 47-56, ISSN: 0065-2598	43

INTERNATIONAL SEARCH REPORT

national application No.
PCT/DK 00/00289

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 42,43
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.: 41,42
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-40, 44-52

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims Nos.: 42,43

Claims 42,43 relate to a human or animal body by therapy/diagnostic methods practised on the human or animal body See PCT Rule. 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds claimed.

Claim 2 relates to a very large number of possible polynucleotides (not counting mismatches due to medium stringency), at least 7322436 polynucleotides, most of them not having the function of the complete sequence, SEQ ID NO:1. The search has been limited to polynucleotides being homologous to the complete SEQ ID NO:1, since the application, provides support within the meaning of Article 6 PCT or disclosure within the meaning of Article 5 PCT for SEQ ID NO 1 and 2.

Continuation of Box I.2

Claims Nos.: 41,42

Claim 41 relates to compounds, claim 42 relates to their use, identified by any of the methods in claim 32-40.
The claims cover all compounds having this characteristic or property whereas the application provides support within the meaning of Article 6 PCT or disclosure within the meaning of Article 5 PCT for only the compounds of claim 43. In the present case, the claims lack support, and the application lacks disclosure, that a meaningful search over the whole claimed scope is impossible.
Consequently, a search has not been performed for claims 41 and 42.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-40, 44-52

   reveals products, methods and uses related to KCNQ5

2. Claims: 13, 15, 16, 51 and 52

   relates to polypeptides encoded by polynucleotides complementary to SEQ ID NO:1. The polypeptides encoded by polynucleotides complementary to SEQ ID NO: 1 are completely different polypeptides than potassium channels. Therefore, these polypeptides are not linked to KCNQ5 by a special technical feature. To start a search for these polypeptides would require an additional fee. The description does not disclose any function of these polypeptides therefore an additional search could find that invention 2 can be divided into further inventions thus requiring extra additional fees.

3. Claims: 41-43

   relates to chemical compounds identified by the method of claims 32-36. The use of the compounds of claim 43 is known in the art and consequently, the possible compounds of claim 42 and the compounds of claim 43 are not linked by a special technical feature in the meaning of PCT Rule 13.2. In principle, the number of inventions is as large as the number of chemical compounds covered by claim 41. Only claim 43 has been searched in any extent. No additional fee for this invention is requested, see Box I.2.

   An additional fee is required for invention 2.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9907832 A	18-02-1999	AU 8266598 A	01-03-1999
		EP 1007638 A	14-06-2000
WO 9921875 A	06-05-1999	EP 1037900 A	27-09-2000